NC 05539 Nicotiana tabacum Carbohydrate Biochemistry

D. A. Danehower, Project Leader

I. Summary of Research:

A. Introduction:

The year marked the inauguration of this research project. The goals of this project are:

1. To develop an accurate and rapid quantitative and qualitative assay for the saccharide esters (S.E.s) of tobacco using chromatographic techniques.

2. To screen various cultivars for levels of saccharide esters using the methodology developed from the above. This screening will examine S.E.'s as a function of climate, stalk position, cultural practice, and curing regimen.

3. To correlate S.E. content with other leaf surface components such as the duvanoid and labdanoid classes of terpenes.

Our rationale for this project is founded in the significant contribution saccharide esters and their breakdown products make to the aroma of tobacco. It is important that tobacco breeders and agronomists have an accurate method for assessment of the levels of these materials in experimental cultivars so that they can more accurately determine the flavor potential of the plants.

B. Summary of Progress:

Initial research focused on development of chromatographic methods for S.E. analysis. Two parallel paths were followed at this time: a procedure utilizing high performance liquid chromatography (HPLC) and a refractive index detector, and another procedure using capillary gas chromatography (GC) and a flame ionization detector. The first method has the advantage of rapid analysis time, but requires (at this juncture) considerable sample preparation. The second method is essentially an adaptation of methods currently in use in industry. Its advantage is rapid sample preparation and concurrent analysis of other leaf constituents; its disadvantage is long analysis times.

Presently we are using the capillary GC method in our laboratory while continuing experiments designed to minimize sample preparation time for the first method. In this area, we are presently evaluating liquid-liquid partitioning, short column chromatography with normal and reversed phase materials, gel permeation chromatography, and the new "sep-pak" technologies.

Using capillary GC we have examined a number of cultivars for saccharide esters. These include Izmir, Samsum, Cavalla, Galpao, TI 165, TI 1309,

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TI 1068, and TI 698, as well as N. glutinosa, which appears to have S.E.'s of an unusual nature. Izmir tobaccos grown by W. H. Johnson have been sampled twice during the growing season as a function of three stalk positions.
This tobacco has also been subjected to three curing regimens and these materials have been sampled as well. These regimens include classical sun curing as well as bulk curing on and off the stalk. Data from all these experiments has been collected and is currently under analysis in our lab.

C. Other Research:

Several other projects have been initiated in our laboratory. These include examination of a number of cultivars for levels of labdanoids. This project involves either HPLC or GC analysis of leaf washes and quantitation of labdane levels. Research in this area is in support of a breeding project currently being contemplated by Dr. E. A. Wernsman of this department.

Another project underway is a re-examination of materials collected by Dr. J. A. Weybrew during a Phytotron study on the effect of climate on NC 2326, Xanthi Oriental, N. sylvestris, and N. tomentosiformis (the latter two species are progenitors of N. tabacum). This project is utilizing the same capillary G.C. methods as the S.E. research.

II. A. Graduate Students:

None

B. Special Students: Ms. Susan Danchi was employed under the R. J. Reynolds Undergraduate Apprentice Program during the summer of 1983.

III. Postdoctoral Fellows:

None

IV. Publications:

Danehower, D. A. The Chemical Ecology of the Mexican Bean Beetle, Epilachna varivestis (Mulsant) Ph.D. Thesis, North Carolina State University, Raleigh, NC.

Bordner, J., Danehower, D. A., Thacker, J. D., Kennedy, G. G., Stinner, R. E., and Wilson, K. G. Chemical Basis for Host Plant Selection. In: Plant Resistance to Insects. P. A. Hedin, ed. ACS Symposium Series No. 208. Am. Chem. Soc., Washington, DC, 1983, pp. 245-264.

V. Manuscripts Accepted for Publication:

Danehower, D. A., and Bordner, J. Cuticular Wax of Epilachna varivestis. J. Ins. Biochem.

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VI. Manuscipts in Review:

Danehower, D. A., and Bordner, J. Cuticular Hydrocarbons of Epilachna varivestis. J. Ins. Biochem.

Danehower, D. A., and Bordner, J. Cuticular Fatty Acids of Epilachna varivestis (Mulsant) J. Ins. Biochem.

VII. Papers Presented at Professional Meetings:

None

VIII. Graduate Student Theses:

None

IX. Acknowledgements:

The project leader gratefully acknowledges the support and assistance of the following people: Drs. J. A. Weybrew, W. W. Weeks, R. C. Long, E. A. Wernsman, and Anne A. Dudley.

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03245 - PHYSIOLOGICAL INVESTIGATIONS IN FLUE-CURED TOBACCO

R. C. Long

Remobilization of Nitrogen During Growth and Senescence (R. C. Long and R. Goenaga).

The growth of the flue-cured tobacco plant involves the sequential development of 18 to 22 leaves. At topping time, leaves at the base of the plant are beginning to senesce while those near the top are still rapidly expanding. Concomitantly, there is rapid depletion of soil nitrate and the plant will have absorbed over 90% of the N to be absorbed in the above ground tissues, resulting in an apparent N stress during the latter portion of the growing period.

Although it has been assumed that younger, actively growing leaves receive their N from older, senescing leaves, there is no direct evidence to demonstrate that the remobilized N from older leaves is sufficient to sustain the active growth of the younger leaves or whether an intense N turnover occurs among all plant parts.

To test the hypothesis that the remobilization of N from the lower leaves is sufficient to sustain the growth of the upper leaves, a study was initiated to determine: a) the uptake of N after topping and virtual depletion of soil N, b) the accumulation of 15N into different plant parts and node positions after topping, c) the remobilization of N from senescing leaves into younger leaves, and d) the form in which N is remobilized.

The study was conducted at the Central Crops Research Station in 1983 using a block planting of McNair 944 flue-cured tobacco. Standard cultural practices were utilized as much as possible. The soil nitrate level was determined just before topping. Leaching irrigation was then applied to remove the nitrate from the root zone. An equivalent amount of K15NO3 was then applied to the soil surface. At weekly intervals, plants (including roots) were sampled and divided into 11 plant parts. Eight sampling periods and five replications (3 plants each) were used. A total of 440 samples were dried, weighed, and ground, and are being processed for total N and 15N analyses of the NO3, insoluble N, and soluble fractions. A nitrogen balance sheet will be constructed for the weekly samplings to determine how the small, upper leaves are nurtured.

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Rank Tobacco--Manipulating the Ripening Process (R. C. Long and E. Felipe).

At harvest time, flue-cured tobacco farmers commonly experience problems due to excess nitrogen. This problem may arise from several causes: nitrogen over-fertilization, erroneous correction of leached nitrogen, or prolonged utilization of nitrogen resulting from dry weather or drought. No matter the reason, excess nitrogen near the end of the growing cycle delays normal ripening, makes the leaf difficult to cure, and adversely affects chemical and physical quality. Therefore, a study was undertaken in 1983 to identify means of dealing with excess N near the end of the growing cycle. Specific objectives were to: 1) determine the effects of special management procedures on the rate of ripening and harvesting pattern of tobacco grown with excess N; 2) determine the effect of these practices on yield and quality; and 3) apply this knowledge, if these procedures prove effective in modifying the effects of excess N, to the practical situation with particular emphasis given to the time of application and intensity of such procedures.

Two general managerial approaches were used: elimination or reduction of excess nitrogen from the root zone by leaching via overhead irrigation, and reduction in the ability of the plant to absorb nitrogen by reducing the root mass through root pruning and flooding.

The study was conducted at the Central Crops Research Station using McNair 944 planted in a RCB design with four replications. Check treatments of 60 and 90 lbs. N/A were established. Four additional treatments at the 90 lb. N/A level were established: leaching, flooding, root pruning one side of the row, or root pruning two sides of the row.

The N variable was established at the time of lay-by cultivation. The other treatments were established immediately following the first priming. The flooding treatment was implemented by damming the furrows around the plots and flooding for 48 hours. Root pruning was accomplished by pulling a sharp blade through the soil at a depth of 6 inches and about 4 inches from the plant.

Although the analysis of the data are incomplete, the 90 lb. check treatment produced the highest yield while the flooding treatment produced the lowest; the difference probably will not be significant. The 60 lb. check and the leaching treatments produced yields slightly greater than the 60 lb. check. The yields of the root pruning treatments were intermediate to the highest and lowest.

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Three days after the flooding treatment was completed, it was necessary to harvest those plots (priming 2). Thereafter, all treatments were harvested on the same priming schedule.

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Other than the flooding treatment, 4 primings completed the harvest. The whole-plant quality indices for the treatment averages ranged from 55 to 62, the 90 lb. check being the lowest and the flooding treatment the highest. The 60 lb. check tobacco had an index of 60.

In general, the differences in quality index were not as great as had been anticipated. It remains to be seen whether the alkaloid, sugar, and the percent unripe and immature data will be any more informative.

Production of Protein for Tobacco (R. C. Long, T. A. Bartholomew, and A. A. Dudley).

A major concern regarding the use of tobacco as a source of food-grade protein has been potential protein yields and the associated cost of production. Two general approaches have been taken to provide information on these problems: 1) improvements in cultural practices to increase protein yields, and 2) modification of cultural practices to reduce production costs.

Seeding treatments-W. The study conducted at Whiteville consisted of two seeding treatments and four genotypes in a split plot design. The Conventional seeding treatment utilized standard plantbed practices, i.e., methyl bromide fumigation and plastic covers. The Direct-Seeded treatment did not use fumigation or covers; the beds were seeded about two weeks later than the Conventional treatment, sprayed with Enide, and covered lightly with pine straw.

Germination and growth of the Direct-Seeded beds were delayed because of the later seeding date; excellent plant stands were obtained from both treatments, however. As in 1982, five harvests were made in the Conventional as opposed to four in the Direct-Seeded treatment.

Contrary to the 1982 results, hollow stalk rot was a serious problem in the Direct-Seeded treatment; plant populations were reduced an average of 80% following the second harvest. Plant populations were reduced only about 18% in the Direct-Seeded treatment. As a result, biomass yields of the 3rd and subsequent harvests were considerably reduced in the Conventional treatment. TI-560 was particularly hard hit by the disease organism in both treatments.

Granville Wilt was observed in all plots toward the end of the season.

In spite of the presence of hollow stalk rot, biomass yields were very good. Average biomass yields for the four genotypes ranges from 188 to 218 mt/ha (ave. 197) in the Conventional treatment and from 140 to 176 mt/ha (ave. 159) in the Direct-Seeded treatment. It is expected that protein yields will be equally high.

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Genotypes-Ox. Seven genotypes were grown under conventional plantbed conditions at Oxford. Early growth was poor and non-uniform, perhaps because the plastic covers were removed earlier than usual. As a result the biomass yield of the 1st harvest was lower than in previous years. However, a very large yield was obtained at the 2nd harvest. A moderate, but lower than the 1st harvest, yield was obtained at harvest 3. Nonetheless, the season total biomass yields for the genotypes ranged from 96 to 141 mt/ha (ave. 111). Md-609 was the highest with all other genotypes below 112 mt/ha. These yields are comparable to those obtained the previous years at the Oxford location.

Population-Ox. The population study conducted in 1982 was repeated in its entirety in 1983. Seven populations were established by seeding in 4" rows and thinning to 2", 3", 6", 10", 13", 16", and 20" between plants. In addition, a random seeded treatment was established by broadcasting seed over the plot. This resulted in a population of about 1.5 million/acre.

The results obtained in 1983 were very similar to 1982. That is, plant populations changed little between harvests for the 6, 10, 13, 16, and 20" spacings. Populations in the Random, 2, and 3" treatments decreased following each of the first two harvests so that by the 3rd, and final, harvest about twice as many plants/sq. ft. were left compared to the less dense spacings.

Treatment seasonal biomass yields ranged from 103 to 146 mt/ha (ave. 126). This compares well to the yield of 132 mt/ha obtained the previous year.

Covers-Ox. A study using different types of plantbed covers was established with an eye toward reduction of production costs. The soil was fumigated with methyl bromide in the usual way. After seeding, perforated plastic, KCNW, or netting was applied to the plots. The KCNW cover is a new, experimental polypropylene non-woven material weighing 0.8 oz./yd. The netting was a plastic based material with 1.5" squares; this material was placed over a light wheat straw mulch to prevent it from blowing off the plots.

As expected, the plastic cover stimulated the earliest and most rapid growth. The netting and straw cover produced plants considerably slower, with the KCNW cover being intermediate. At the time of the first harvest, the plastic treatment had 29 plants/sq. ft., the KCNW 23, and the netting 16. However, all treatments produced equivalent biomass yields during the season.

The KCNW cover provided an adequate cover for plant production. Soil moisture appeared more uniform and temperatures were more moderate under the KCNW than the plastic. The material was also subject to less tearing than we have noted in the past with other non-woven materials, suggesting that it may be usable for several years.

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Other crop species-Ox. To determine what biomass yields might be expected from other crops, pearl millet (variety 'Tifleaf') and soybean (variety 'Jupiter', maturity group 10) were seeded after the danger of frost had passed. Seed were sown in 8" rows at the rate of 40 lbs/acre, respectively. The plots were fertilized and handled similar to the tobacco plots. When the plants were about 30" tall, they were harvested at about 9" above the ground, fertilized, and allowed to grow a new crop. Four harvests were obtained from the millet, yielding a total biomass of 85 mt/ha. Only two harvests were secured from the soybeans before frost; the yield was approximately 30 mt/ha.

Tillage-Ox. A study was conducted to compare different types of tillage on the germination and growth of tobacco when seeded without methyl bromide fumigation or covers. Plots that had been laid out the previous fall and seeded with wheat were treated with Paraquat in mid-April to kill the vegetation. This operation had been delayed because of wet weather. On the same day, 2500 lb./A of 12-6-6 fertilizer were applied; 1.5 lb. a.i./A Enide were applied to the minimum and No-Tillage treatments. The tillage treatments were established ten days later.

For the Normal tillage treatment, four passes were made with a til-rovator followed by one pass with a cultipacker. A Minimum Tillage treatment was provided by cutting the wheat to a height of 4" with a rotary mower before making one pass with a 6 ft. double-gang finishing disc set in the open position. The No-Tillage treatment was established by mowing the wheat to a height of 4". All plots (64" x 15') were then seeded with 1 gram seed in lime and sprayed with 0.5 lb. a.i./A Ridomil. After seeding, a cultipacker was pulled over the Minimum and No-Tillage plots one time. Wheat straw was applied to the Normal treatment.

Each of the tillage treatments was further divided into three herbicide treatments. Enide (4.5 lb. a.i./A in the minimum and No-Tillage treatments; 6 lb. a.i./A in the Normal Tillage treatment) was applied to two of the beds on the day of seeding. Three weeks later after the seed had germinated, an additional application of Enide (6 lb. a.i./A) was applied to one of the beds to which Enide had been previously applied; this provided 1X and 2X rates of the Enide treatment. Devrinol (1.0 lb. a.i./A) was applied to the third bed in each of the tillage treatments. Each treatment combination was replicated three times.

Although germination was slow in all tillage treatments, it was particularly poor in the Normal treatment. It did appear, however, that the stubble mulch provided by the Minimum and No-Tillage treatments was beneficial to germination by providing protection from drying winds and low temperatures. Although the herbicides gave good initial weed control, weeds (especially ragweed) began to assert themselves after a few weeks and plant growth was slow. Weeds were so heavy they precluded taking the first harvest with the mechanical harvester.

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In summary, attempts to use the direct seeding concept (i.e., no methyl bromide fumigation or covers) with various tillage and herbicide combinations met with failure in both 1982 and 1983. It is tempting to speculate that the early spring temperatures fluctuate sufficiently as to retard early growth of the tobacco but not that of weeds. Consequently, a canopy of tobacco plants does not become established in time to provide competition for the weeds. Yet, the concept has been used successfully at the Whiteville location, perhaps because of less widely fluctuating early spring temperatures and reduced weed pressures.

GRADUATE STUDENTS:

Bartholomew, T. A. (U.S.A.), M.S., under R. C. Long
Felipe, E. (Venezuela), M.S., under R. C. Long
Goenaga, R. J. (U.S.A.), Ph.D., under R. C. Long and R. J. Volk

PUBLICATIONS:

Black, R. A., W. K. Collins, R. C. Long, and H. Seltmann. 1983. Responsiveness of flue-cured tobacco varieties to ethephon. Tob. Sci. 27:30-34.

Long, R. C. 1984. Edible tobacco protein--moving beyond theory. Crops and Soils 36(5):13-15.

Weybrew, J. A., W. A. W. Ismail, and R. C. Long. 1983. The cultural management of flue-cured tobacco quality. Tob. Sci. 27:56-61.

Long, R. C., and J. A. Weybrew. 1982. Effect of moisture management on flue-cured tobacco quality. 19th Annual N. C. Irrigation Conference Proceedings. pp. 9-19.

MANUSCRIPTS ACCEPTED FOR PUBLICATION:

Beatson, R. A., E. A. Wernsman, and R. C. Long. Divergent mass selection for carotenoids in a flue-cured tobacco population. Crop Sci. (in press).

PAPERS PRESENTED AT PROFESSIONAL MEETINGS:

Bartholomew, T. A., and R. C. Long. Effect of plant population on biomass and protein production of tobacco grown for protein. 30th Tobacco Workers' Conference, Williamsburg, Jan. 11-13, 1983.

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05149 - IMPROVING AND AUTOMATING ANALYTICAL METHODOLOGIES REQUIRED FOR EXPERIMENTAL TOBACCO

W. W. Weeks

The tobacco analytical laboratory has completed analyses of flue-cured samples from the 1983 crop. This year's crop included 15,000 analyses including reducing sugars and total alkaloids. Four hundred samples were determined for total particulate matter and total alkaloids. These analyses were completed by February 15, 1984.

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03837 - EVALUATION OF VOLATILE COMPOUNDS OF TOBACCOS SELECTED FOR DIFFERENT ALKALOID CONCENTRATIONS

W. W. Weeks

Changes in Surface Chemistry and Volatile Oils During Flue-Curing (K. W. Chang, W. W. Weeks, and J. A. Weybrew).

The source of the samples used in this investigation was normally cultured plants of the flue-cured variety NC 2326 (Nicotiana tabacum L.) grown at the Oxford Tobacco Research Station in 1981. A single harvest of approximately 25,000 ripe leaves from node positions 10-12 provided the analytical samples. The leaves not used as at-harvest samples were racked (approximate 500 leaves per rack) for normal flue-curing in small bulk barns.

The stickiness of the trichome exudate was utilized for the collection of "pure" though not-quantitative collection of glandular trichome heads. Fourteen sponge held-down rollers from a conveyer system were encased with preextracted cheesecloth and 8,000 leaves were individually passed face up under these rollers, some of the trichomes adhered to the cheesecloth and were torn off. The leaves were retrieved at the exit end, inverted, and passed through a second time for additional collection from the under surface. The trichome laden cheesecloths were removed and halved longitudinally. One set of cheesecloth halves (GR-T) was stored at 17 degrees C for analysis later. The other set was hung in the curing barn for "yellowing" (to 54.5 degrees C), then stored at -17 degrees C (GY-T).

A second collection of trichomes was made from 8,000 leaves removed from the curing barn after yellowing (Figure 1). Again the laden cheesecloths were halved; one set was stored (YE-T), the other set was returned to the barn for fixing and drying (54.5 degrees C - 71.0 degrees C; CU-T) (Figure 2).

A Varian 3700 gas chromatograph equipped with FID detector and a CDS 111 data processor was used to obtain the analytical data from this study. To avoid destruction of the glass capillary columns, the samples were screened on a 1/8" x 10' nickel Carbowax 20 M packed column to determine proper analytical aliquot for capillary chromatography.

In preparation for GC analysis (Figure 3), each set of cheesecloths was extracted with chloroform in a large glass column equipped with a teflon stopcock. The chloroform was filtered through celite to remove debri and concentrated to about 500 ml. The concentrate was washed with 1M tartaric acid for

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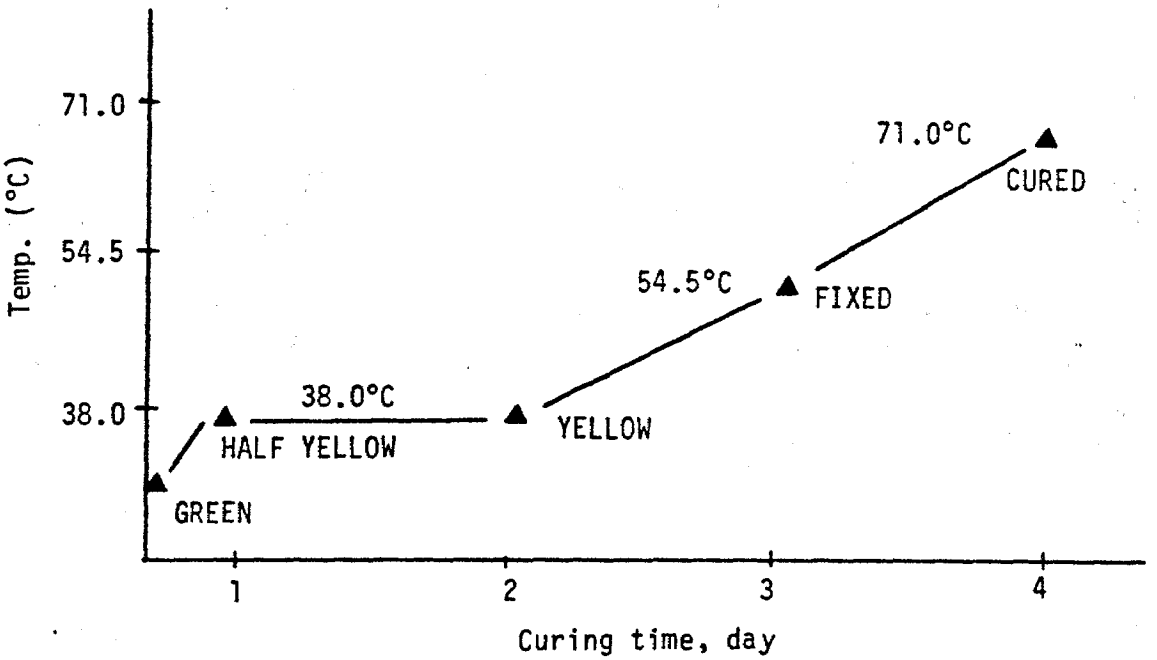

Figure 1. Sampling steps for duvane and volatile oils during curing.

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			Codes
Trichomes			
At harvest	8000 ripe lvs.	trichomes on cheesecloths — stored at -17° C	GR-T
		trichomes on cheesecloths — cured 67 hrs	GY-T
Yellowed	8000 lvs.	trichomes on cheesecloths — extract ($CHCl_3$)	YE-T
		trichomes on cheesecloths — cured	YC-T
Surface Resins			
At harvest	100 lvs.	washed with $CHCl_3$	GR-S
Half-Yellowed	100 lvs.	washed with $CHCl_3$	HY-S
Yellowed	100 lvs.	washed with $CHCl_3$	YE-S
Fixed	100 lvs.	washed with $CHCl_3$	FX-S
Cured	100 lvs.	washed with $CHCl_3$	CU-S
Volatile Oils			
At harvest	25 lvs.	steam distilled	GR-V
Half-Yellowed	25 lvs.	steam distilled	HY-V
Yellowed	25 lvs.	steam distilled	YE-V
Fixed	25 lvs.	steam distilled	FX-V
Cured	25 lvs.	steam distilled	CU-V

Figure 2. Collection and codings of experimental samples

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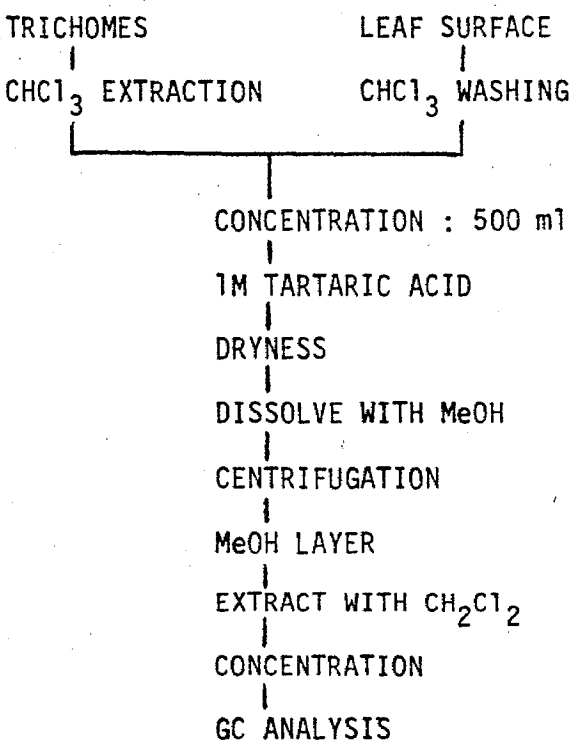

Figure 3. Extraction and preparation of extracts for GC analysis.

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denicotinization and concentrated to dryness in a rotary vacuum evaporator. Duvane compounds in the residue after evaporation were selectively extracted with methanol, leaving behind the high molecular weight cuticular hydrocarbons; the insoluble waxes and other debris were discarded. The methanol layer was mixed with water 50/50 and saturated with NaCl and extracted with CH2Cl2. The CH2Cl2 solution was evaporated under vacuum at 20 degrees C until most of the solvent disappeared. The viscous solute was further evaporated under nitrogen until the solvent vapor completely vanished. Following this preparation, each sample was carefully weighed and the unit of the weight was used as the basis for the quantitative comparisons of individual duvane compounds between treatments. Finally the solutes were dissolved in 1 ml CH2Cl2. One µl was injected in capillary GC column (Figure 3).

Surface extraction of leaves were prepared for comparison with excised trichomes. One hundred leaves at-harvest were individually immersed in chloroform for 30 seconds. Chloroform extraction of green leaves even for a short period of time removed surface waxes, resins, and other cellular constituents from the leaf surface along with the contents of the trichomes. The complexity of the surface extraction necessitated considerable cleanup before analysis could be performed. The chloroform extract (GR-S) was processed as shown in Figure 3. Other 100-leaf samples removed from curing at half-yellowed (HY-S), yellowed (YE-S), fixed (FX-S) and at the end of curing (CU-S) were similarly extracted (Figure 3).

The trichome samples underwent marked changes during curing. These changes are demonstrated in Figures 4, 5, and 6. The heads of the green mature trichomes appeared clear and translucent but underwent color change directly associated with chemical change (Figures 4.A, 5.A, and 6). Close examination of the excrescence collected on the cheesecloths (Figure 4.B, 5.B) indicated that primarily only trichome heads had been collected. This was most likely because of the stickiness of the heads.

The chromatographic profiles of duvanes in tobacco trichomes and leaf surface contained 72 peaks (Figures 7 and 8). Extracts from surface washings of green leaves and from fixed leaves were submitted to R. J. Reynolds Tobacco Company for GC/MS analysis. The GC column effluent was simultaneously vented into the mass spectrometer for the mass spectrum of each GC peak. From the mass spectra data of 72 compounds molecular structures, 16 compounds were identified by comparison with the mass spectra from the library established by Reynolds Company from previous isolations (Table 1). The structures of many of the other peaks were similar to those identified but structures were not confirmed.

Table 1 exhibits retention times, tentative structures, and quantitative data of compounds isolated both from the trichomes collected on cheesecloths and from extracts of the surfaces of green tobacco leaves. The data are arrayed to illustrate easily

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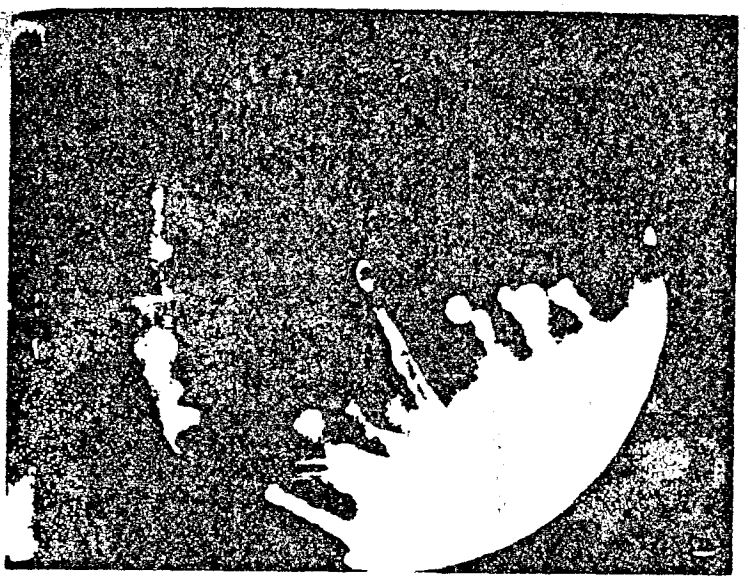

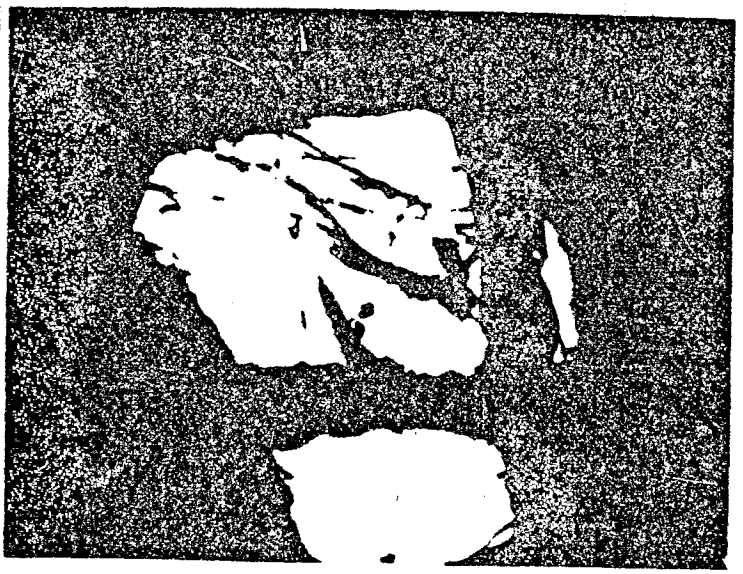

Figure 4.A. Green trichomes on the green tobacco leaf surface of NC 2326.

B. Green sticky trichome substance collected on cheesecloths.

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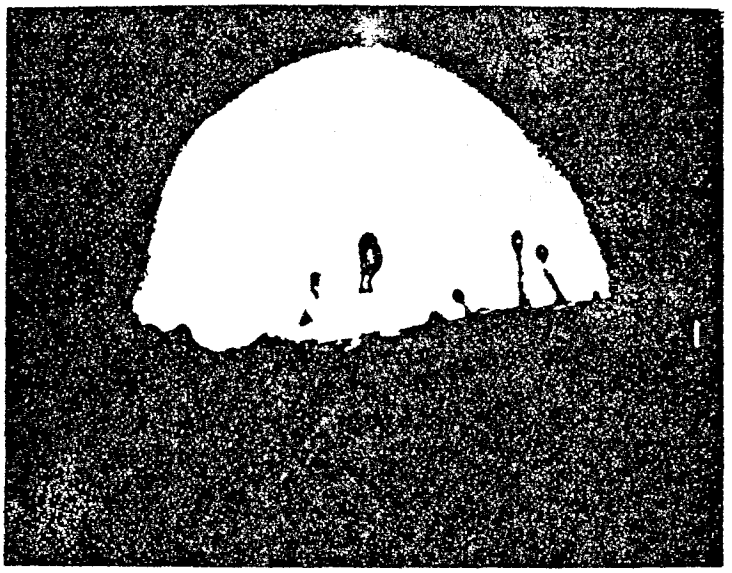

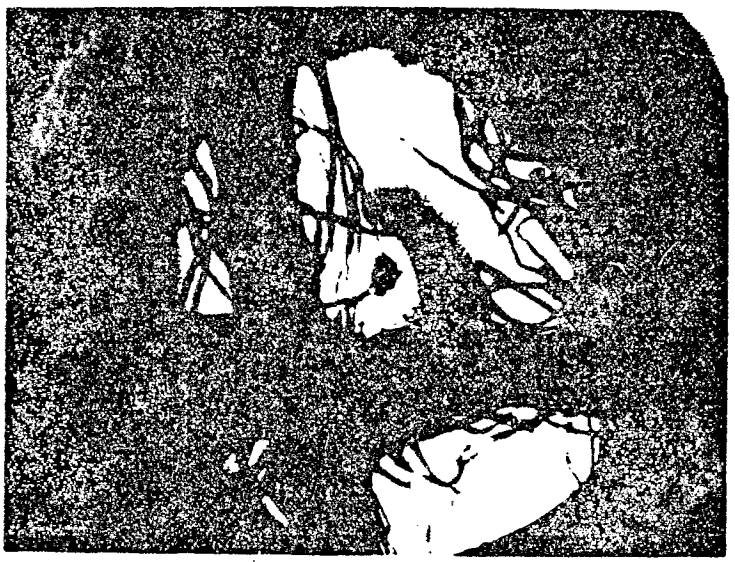

Figure 5.A. Yellow trichomes on the tobacco leaf surface yellowed in the barn.

B. Yellow sticky trichome substance collected on cheesecloths.

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Figure 6. Brown trichomes on the tobacco leaf surface at fixed stage of curing.

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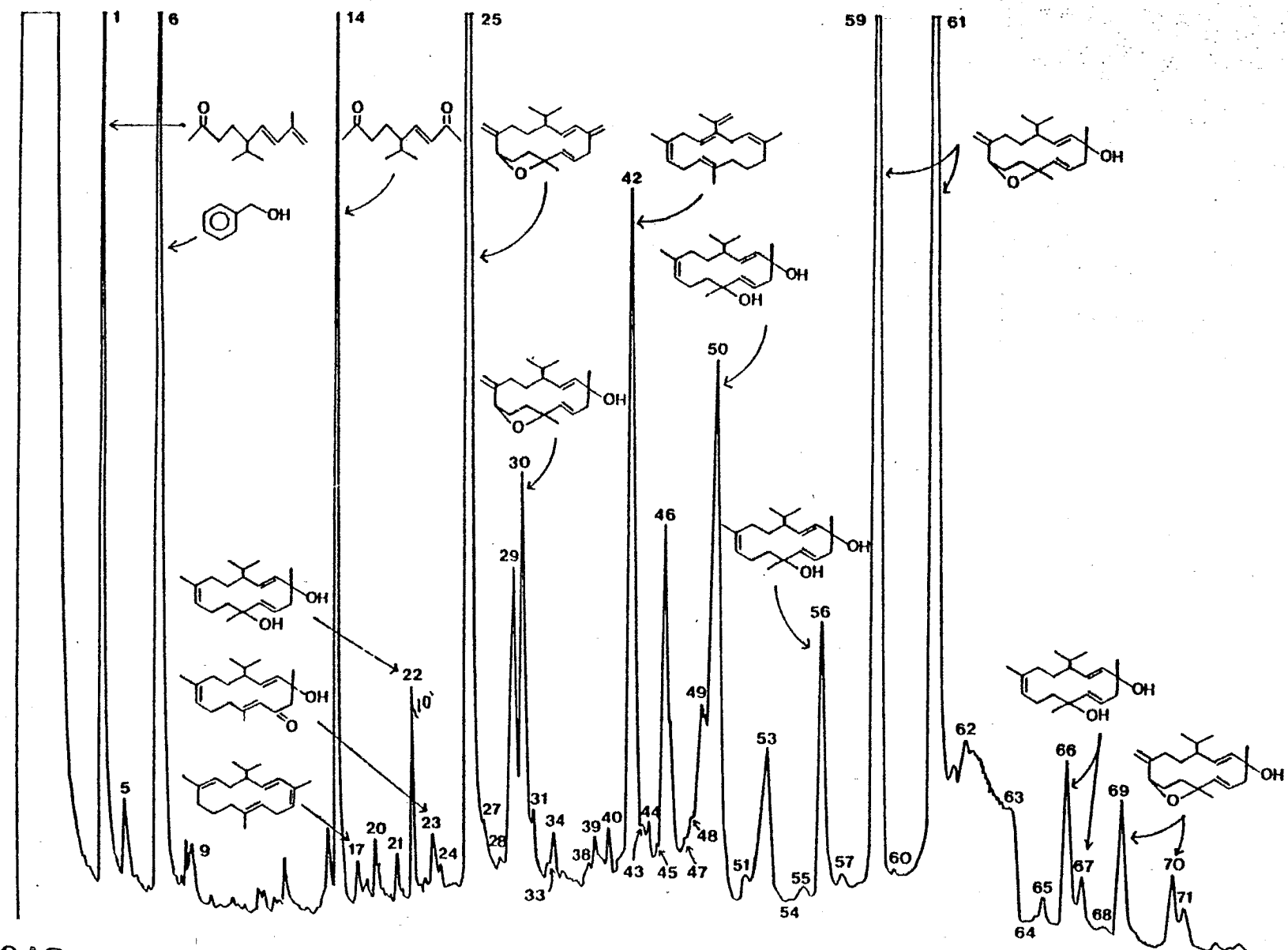

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Figure 7. GC chromatogram of duvane compounds of green tobacco trichomes.

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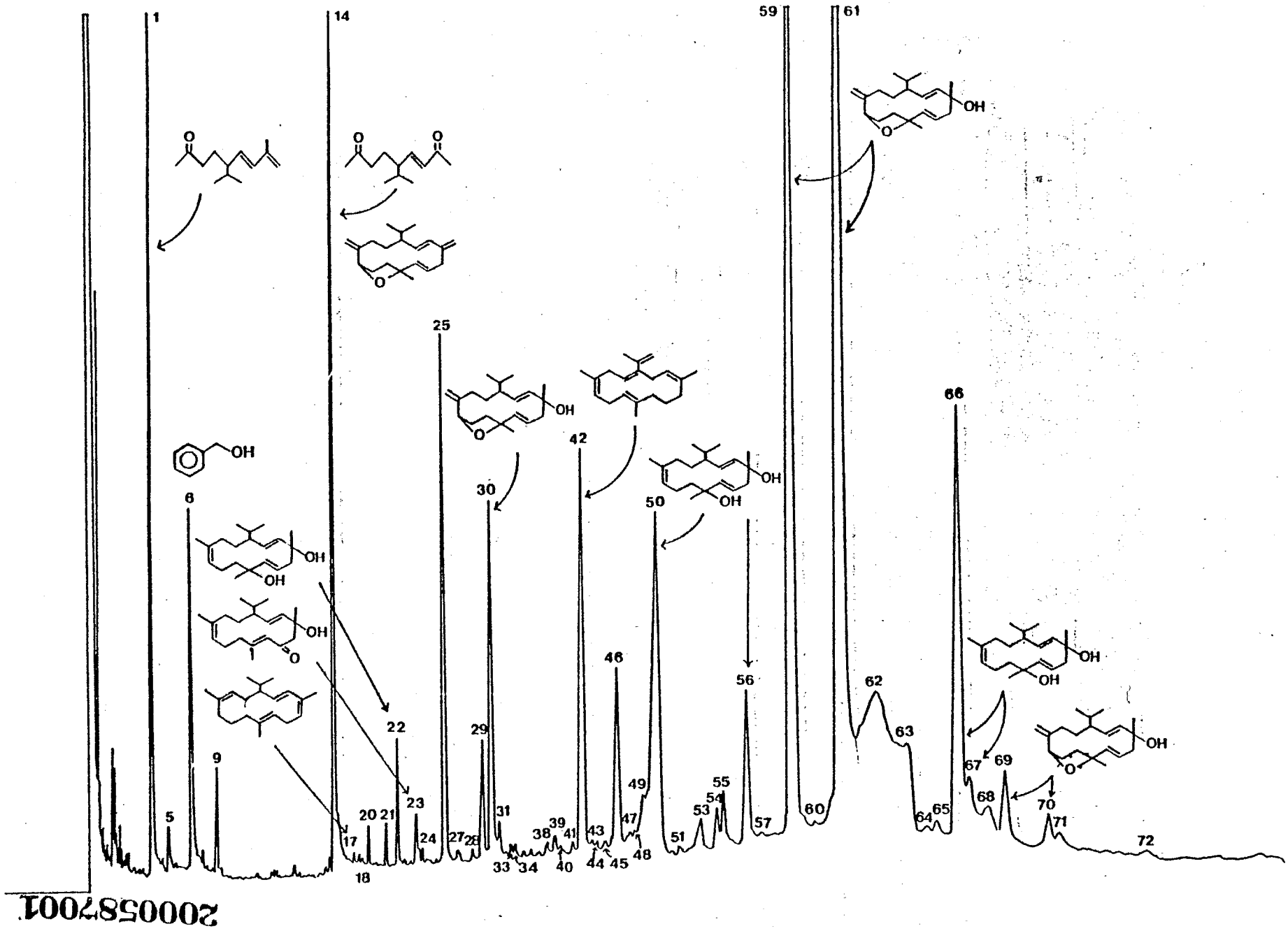

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Figure 8. GC chromatogram of duvane compounds of green tobacco leaf surface

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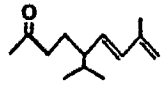
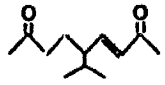
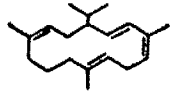
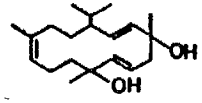
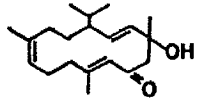
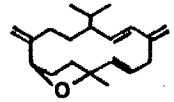
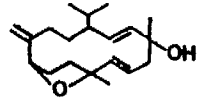
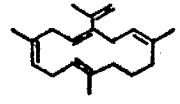
Table 1. Concentration of duvanes and their decomposed components in tobacco surface and trichomes.

unit:mg/g

GC No.	R. T. Min.	Name	Structure	Formula M. W.	References	Surface					Trichome			
						GR-S	HY-S	YE-S	FX-S	CU-S	GR-T	GY-T	YE-T	YC-T
1	10.5	Solanone		$C_{13}H_{22}O$ 194	24,25,28, 29,32	7.17	10.59	11.52	12.93	8.95	7.40	3.89	5.46	5.20
14	31.8	Oxysolanone		$C_{12}H_{20}O_2$ 196	28,29,32	9.03	6.49	7.59	8.50	7.42	7.04	5.49	6.42	5.50
17	33.5	1,4,9,13-Duvatetraene		$C_{20}H_{32}$ 272		0.32	0.15	0.24	0.18	0.21	0.63	0.41	0.60	0.28
22	39.2	3,8,13-Duvatriene-1,5-diol		$C_{20}H_{34}O_2$ 306	29,30,40, 41,45	1.39	0.57	1.31	1.16	0.25	1.99	1.41	0.64	0.53
23	41.4	4,8,13-Duvatriene-1-ol-3-one		$C_{20}H_{32}O_2$ 304	24,25,42, 45,46	0.80	1.41	1.23	0.93	0.41	1.10	0.95	1.21	0.66
25	44.7	5,8-Oxido-1,9-dimethylene-3,13-duvadiene		$C_{20}H_{30}O$ 286	46	6.76	24.17	17.83	12.49	6.99	19.89	15.60	18.24	8.55
30	50.2	5,8-Oxido-9-methylene-3,13-duvadiene-1-ol		$C_{20}H_{32}O_2$ 304	46	7.17	7.21	5.44	5.04	2.07	6.44	6.32	5.36	3.04
42	61.1	12-Isopropylene-5,8, 11,14-uvatetraene		$C_{20}H_{30}$ 270		9.50	28.37	12.33	9.47	3.74	13.37	10.20	8.16	5.33

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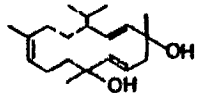
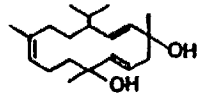
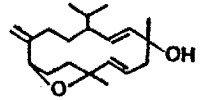
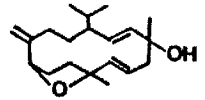
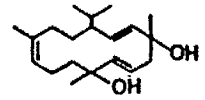
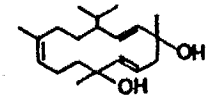
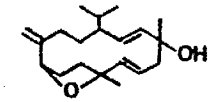
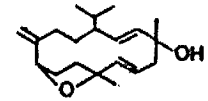
Source: https://www.industrydocuments.ucsf.edu/docs/xtyk0000

Table 1 (continued)

GC No.	R. T. Min.	Name	Structure	Formula M. W.	References	Surface					Trichome			
						GR-S	HY-S	YE-S	FX-S	CU-S	GR-T	GY-T	YE-T	YC-T
50	69.7	3,8,13-Duvatriene-1,5-diol		$C_{20}H_{34}O_2$	29,30,40, 41,45	16.54	22.34	16.59	13.53	4.76	13.44	16.81	19.17	5.94
56	79.9	3,8,13-Duvatriene-1,5-diol		$C_{20}H_{34}O_2$ 306	29,30,40, 41,45	6.62	8.92	9.22	7.86	1.29	5.73	7.44	15.08	2.10
59	85.7	5,8-Oxido-9-methylene-3,13-duvadiene-1-ol		$C_{20}H_{30}O_2$ 304	46	47.85	21.48	45.22	37.55	6.58	25.08	23.80	29.35	9.07
61	91.3	5,8-Oxido-9-methylene-3,13-duvadiene-1-ol		$C_{20}H_{32}O_2$ 304	46	71.29	38.98	57.39	49.63	9.58	31.19	34.97	45.97	10.80
66	104.4	3,8,13-Duvatriene-1,5-diol		$C_{20}H_{34}O_2$ 306	29,30,40, 41,45	24.29	6.78	13.77	11.96	1.32	3.46	4.18	3.48	1.73
67	105.5	3,8,13-Duvatriene-1,5-diol		$C_{20}H_{34}O_2$ 306	29,30,40, 41,45	5.04	3.31	3.37	3.39	0.69	1.48	1.78	1.69	0.81
69	109.6	5,8-Oxido-9-methylene-3,13-duvadiene-1-ol		$C_{20}H_{32}O_2$ 304	46	6.10	3.42	5.73	5.47	2.88	3.37	4.20	3.90	3.10
70	114.6	5,8-Oxido-9-methylene-3,13-duvadiene-1-ol		$C_{20}H_{32}O_2$ 304	46	2.37	3.12	2.56	2.96	14.82	1.87	4.61	6.81	8.19

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Source: https://www.industrydocuments.ucsf.edu/docs/xtyk0000

all combinations of samples from green leaves and trichomes, and show the changes that occur in the duvanes during curing.

The oxidative form of duvane and an allylic isomer of cembrene, 1,4,9,13-duvatetraene (Peak 17), was found in trichomes and in the leaf surface washings. Fluctuations in concentrations of surface and trichomes during curing are represented in Table 1. Higher quantities of 1,4,9,13-duvatetraene were found in green and yellow trichomes than in green and yellow surface samples. The concentration of 1,4,9,13-duvatetraene of the green and yellow trichomes collected on cheesecloths (GR-T and YE-T) decreased significantly during curing, while the decrease of the concentration of this compound in the surface washing was not significant.

Solanone (Peak 1) in the surface extract of leaves increased from green to fixed leaves, but decreased during the drying stages of curing. In the case of trichome extracts, the amount of solanone from green leaf was higher than that from the yellowed leaf and decreased further during the later stages of curing.

The higher concentrations of solanone in leaf surface extracts than in excised trichomes possibly suggests that exudate from trichomes had flowed to leaf surface.

Oxysolanone (Peak 14) also, a duvane derivative, contributes heavy carrot-like flavor to tobacco and adds sweet ketonic flavor to smoke. Changes in the concentration of oxysolanone are shown in Table 1. Again, the higher concentration in surface extracts may be the result of exudation from trichomes.

The differences in the quantities of solanone, oxysolanone, and total duvanes between surface and trichome samples at green, half-yellow, yellow, fixed, and cured stage are summarized in Table 2. The total amount of all compounds in general were higher in surface than in trichomes in all samples.

The duvanes alone appear to be of little value to tobacco aroma but the compounds that are derived from the duvane compounds are of considerable importance. From this study, solanone and oxysolanone were the two compounds quantified that were associated.

Further evaluation of the aroma associated with the total tobacco leaf to add to the contribution from surface components was made of the curing and cured leaves during this study. These studies were made by capillary GC analysis of steam distillation of 25 leaves.

The GC profile of the distillate from the yellowed tobacco is reproduced in Figure 9 and shows 242 distinct peaks. Seventy two compounds with peak areas greater than 100,000 on the CDS 111 integrater units were identified by GC/MS and quantified. These 72 compounds were classified by functional groups and from this

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Source: https://www.industrydocuments.ucsf.edu/docs/xtyk0000

Table 2. Comparisons of the changes in the concentrations of solanone, oxysolanone, and total duvanes in extracts of isolated trichomes versus surface washing of intact leaves during curing.

unit : mg/g

Treatments / Compounds	Green		Half-yellow	Yellow			Fixed	Cured	
	GR-S	GR-T	HY-S	YE-S	YE-T	GY-T	FX-S	CU-S	YC-T
Solanone	7.2	7.4	10.6	11.5	5.5	3.9	12.9	9.0	5.2
Oxysolanone	9.0	7.0	6.5	7.6	6.4	5.5	8.5	7.4	5.5
Total duvanes	206.1	129.1	170.1	192.1	159.7	132.7	161.6	55.6	60.2

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Source: https://www.industrydocuments.ucsf.edu/docs/xtyk0000

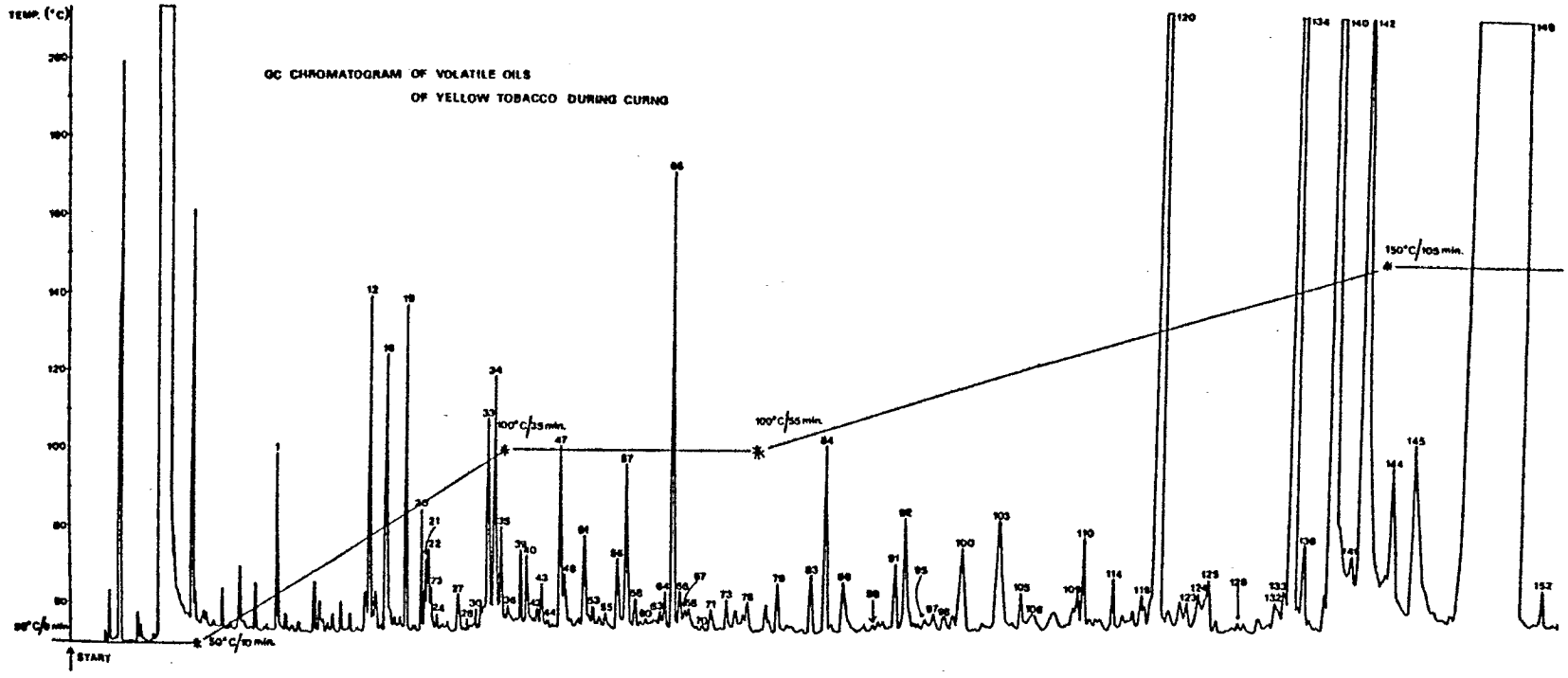

Figure 9. GC chromatogram of volatile oils of yellow tobacco during curing.

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Source: https://www.industrydocuments.ucsf.edu/docs/xtyk0000

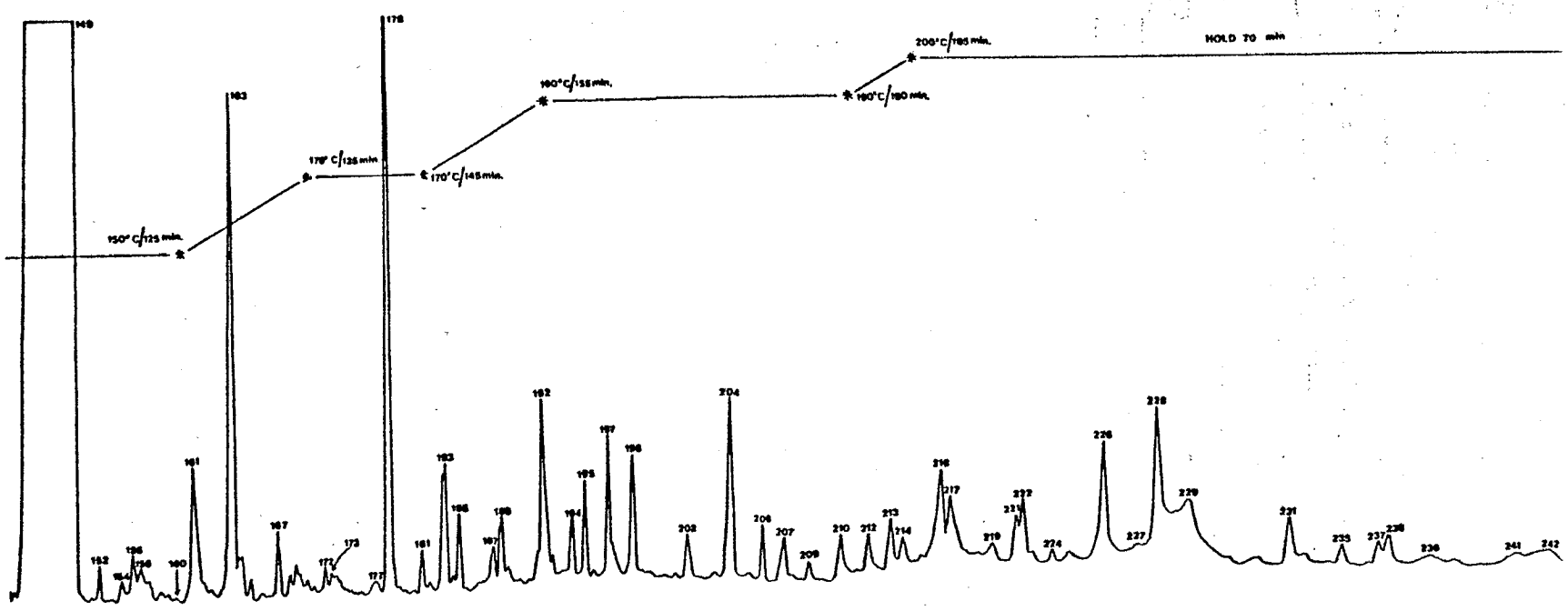

Figure 9 (continued)

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Source: https://www.industrydocuments.ucsf.edu/docs/xtyk0000

tentative source. Many of these compounds have structures suggesting them to be degradation products of carotenoids, duvanes, lignin, lipids, and chlorophyll.

Compounds related to Carotenoids. Oxygenation of carotenoids occurs during senescense, curing and aging of tobacco. Carotenes are C40 polyunsaturated hydrocarbons. Eighteen carotenoids are known of which lutein and β-carotene are the two primary ones. The most important degradations of carotenoids are oxidations which may occur by photosensitized oxidation, autooxidation, or enzymatic oxidation. Enzymatic oxidation occurs in the intact plant and results in breakdown of β-carotene to C9, C11, C13 cyclic and aliphatic oxygen containing compounds.

Further oxidative degradations of carotene compounds occur during fermentation and aging and are directly dependent upon molecular oxygen. For example, β-carotene is considered a precursor of mariolide (Peak 198), β-ionone, 6-hydroxy-2,2,6-trimethyl-cyclohexanone, geronic acid, damascenone (Peak 134, geranylacetate, megastigmatriene-one isomers (Peaks 178-2, 195) and others.

Cleavage of the terminal cyclic groups of the β-carotene molecule gives mariolide, Peak 198, or alternatively, it might be formed via the oxygenation of β-ionone. These oxygenated compounds are reported to be flavor compounds in tea, food, and tobacco smoke. A slight "cooling" effect in smoke has been associated with the addition of mariolide and similar compounds to tobacco. The quantities of mariolide found in fresh green tobacco are very small. The concentration of this compound increased three-fold from green to fixed tobacco and then decreased during the final stage of curing.

Xanthophylls are degraded by photosensitized oxidation to neoxanthins. Neoxanthins are responsible for very important oxygenated compounds in tobacco. Damascone (Peak 133), β-damascenone (Peak 134), and 4-hydroxy-β-damascone (Peak 216) in the chromatographic profile were found in the distillate of volatile oils. When added onto tobacco, they impart a floral-like aroma and add "body" to the smoke. A further description of damascenone has been identified as burley-like aroma. In this study, damascenone was considered to be a significant component.

Ohloff, G (Classification and Genesis of Food Flavors; The Flavor Industry, Volume 3, 501-508, 1972) showed the possible formation of oxygen-containing fragments from a partially hydrogenated carotenoid model (Figure 10). The aliphatic and monocyclic isoprenoid flavor compounds are formed by fragmentation of the oxidation products of the corresponding carotenoids. Nineteen tobacco volatiles have formed by oxidative cleavage of the 6-7, 7-8, 8-9, or 9-10 bonds of appropriate cyclic tobacco carotenoids (Figure 10). This scheme shows the formation of C13, C11, C10, and C9 products as cyclic compounds, and acetone, 6-methyl-5-hepten-2-one (Peak 39), geranyl acetone

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Source: https://www.industrydocuments.ucsf.edu/docs/xtyk0000

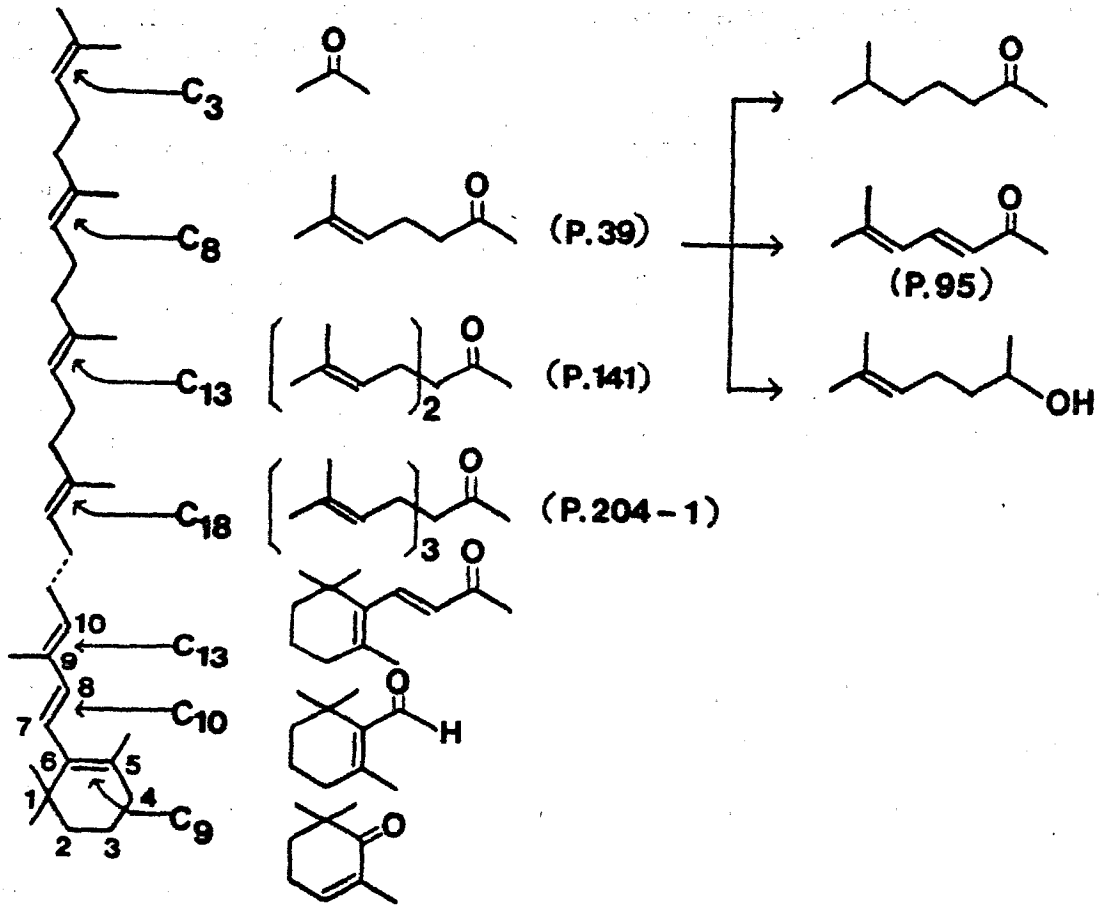

Figure 10. Oxidative cleavage of the olefinic chain connecting the terminal cyclic structures of carotenoids.

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Source: https://www.industrydocuments.ucsf.edu/docs/xtyk0000

(Peak 141), and farnesyl acetone (Peak 204-1) as aliphatic compounds from β-carotene.

Ten C13 - derivatives of carotenoids -- 4,6,8-megastigmatriene-3-ones (Peak 178-2, 195), 3-oxo-α-ionol (Peak 228), 1,3,7,7-tetramethyl-9-oxo-2-oxabicyclo-[4.4.0]-dec-5-ene (Peak 155), damascone (Peak 133), 3-hydroxydamascone (Peak 216), dehydro-β-ionone (Peak 173), 4-hydroxy-β-ionol (Peak 229), 3-hydroxy-β-ionol (Peak 231), and damascenone (Peak 134) were identified in this study. Other derivatives of caroentoids were mariolide (Peak 198; C11) and isophorone or 4-ketoisophorone (Peak 109; C9).

The four 4,6,8-megastigmatriene-3-ones differ in the orientation of the substituents on the side chain. The isomers were not identified with retention times but spectra data of two isomers were identified at 142.17 (Peak 178-2) and 158.22 minutes (Peak 195). 4,6,8-Megastigmatriene-3-one isomers can be formed by dehydration of 4-oxo-α-ionol (Peak 228) during the steam distillation of the tobacco. The megastigmatriene-ones are reported to add body and peppery flavor to smoke.

Changes in the concentration of the megastigmatriene-one isomers (Peak 195; retention time 158.22 min.) occurred during curing and 4-oxo-α-ionol (Peak 228) formed from 3-hydroxy-α-ionone which is produced by cleavage of 9-10 double bond of lutein. The pyran, 1,3,7,7-tetramethyl-9-oxo-2-oxabicyclo-[4.4.0]-dec-5-ene (Peak 155), is derived from cyclization and isomerization of 4-oxo-α-ionol. In this study, the concentration of 1,3,7,7-tetramethyl-9-oxo-2-oxabicyclo-[4.4.0]-dec-5-ene (Peak 155) increased during curing while 4-oxo-α-ionol (Peak 228) decreased. These data suggest that 4-oxo-α-ionol is an intermediate in the formation of 1,3,7,7-tetramethyl-9-oxo-2-oxabicyclo-[4.4.0]-dec-5-ene (Peak 155).

A C9 compound, 4-ketoisophorone or oxophorone (Peak 109), is the product of the oxidative degradation of 6-7 bond of tobacco carotenoids. This compound contributes sweetness and an ionone-like character to smoke. Its concentration increased steadily during curing.

The olefinic chain connecting the terminal cyclic structures of carotenoids is a likely precursor of several aliphatic and olefinic essential oils of tobacco. Acetone, 6-methyl-5-hepten-2-one (Peak 39), geranyl acetone (Peak 141), and farnesyl acetone (Peak 204-1) might have formed by the cleavage of a partially hydrogenated carotenoid as shown in Figure 10. While the concentrations of Peak 39, 141, and 204-1 are low and showed no definite trends over the curing times in this study each has been described as contributing flavor to smoke.

The slight increases in the concentration of linalool oxide, a minor constituent among the volatile oils of tobacco. This lactone (Peak 57) is an oxidative product of 4-methyl-4-hydroxy-5-hexenioc acid, commonly identified as linalool oxide.

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Source: https://www.industrydocuments.ucsf.edu/docs/xtyk0000

Compounds Related to Phenylalanine. The structures of benzaldehyde (Peak 79), benzyl alcohol (Peak 142), phenethyl alcohol (Peak 145), phenol (Peak 156), and p-cresol (Peak 168) suggest that their precursor may have been phenylalanine by the scheme Figure 11. These compounds were found in all samples at harvest and during curing. Benzaldehyde (Peak 79) might be produced from cinnamic acid which is a deamination product of phenylalanine. Benzaldehyde has a sweet and fruity odor and enhances tobacco aroma while phenol adds a sweet and medicinal taste to smoke. The concentration of benzaldehyde (Peak 79) was relatively high at the green, fixed, and cured stages, but was quite low at half-yellow stages. Benzaldehyde may be oxidized to phenol (Peak 156). However, many types of compounds may act as potential phenol precursors through alternative pathways. The changes in the concentration of phenol (Peak 156) tended to increase during curing. The concentration of p-cresol was higher in cured leaf than green leaf, but could not be detected at half-yellow and fixed stages during curing. Benzaldehyde may convert to benzyl alcohol (Peak 142) by the reduction of benzaldehyde.

Also phenylalanine has alternative pathway to form phenethyl alcohol (Peak 145) via phenylacetaldehyde produced by Strecker degradation. These alcohols have characteristic aroma of rose and add floral flavor to smoke. In this study, benzyl alcohol and phenethyl alcohol are abundant neutral constituents; changes in their concentrations during curing and aging are well known.

Compounds Related to Lipids. Lipids are decomposed to volatile components by the action of light, air, and/or enzymes. Through one or another of several pathways, certain lipids convert to simple acids, alcohols, aldehydes, ketones, and esters. For example, 1-penten-3-ol (Peak 28) appears to be a degradation product of linolenic acid.

Among the volatile oil constituents identified in this research, three acyclic carboxylic acids, 3-methyl butanoic acids (Peak 105-1), 2-methyl butanoic acids (Peak 105-2), and hexanoic acid (Peak 140); one alcohol, 1-penten-3-ol (Peak 28); two acyclic aldehydes, 2-methyl propenal (Peak 22) and 2,4-hexadienal (Peak 73); and three esters, ethylene acetate (Peak 66), farnesyl acetate (Peak 181), and methyl linolenate (Peak 222), are the products of lipid metabolism.

2-Methyl propenal (Peak 22) and methyl linolenate (Peak 222) showed decreases in concentration from green to cured leaf, while the concentrations of ethylene acetate (Peak 66) and 2,4-hexadienal (Peak 73) did not change appreciably during curing. 3-Methyl butanoic acid Peak 105-1 plus 2-methyl butanoic acid (Peak 105-2) and farnesyl acetate (Peak 181) increased during curing. Hexanoic acid (Peak 140) generally tended to decrease during curing but was highest at the yellowed stage.

Compounds Related to Chlorophyll. Senescence of a tobacco leaf starts to initiate numerous complex metabolic reactions.

2000587011

Source: https://www.industrydocuments.ucsf.edu/docs/xtyk0000

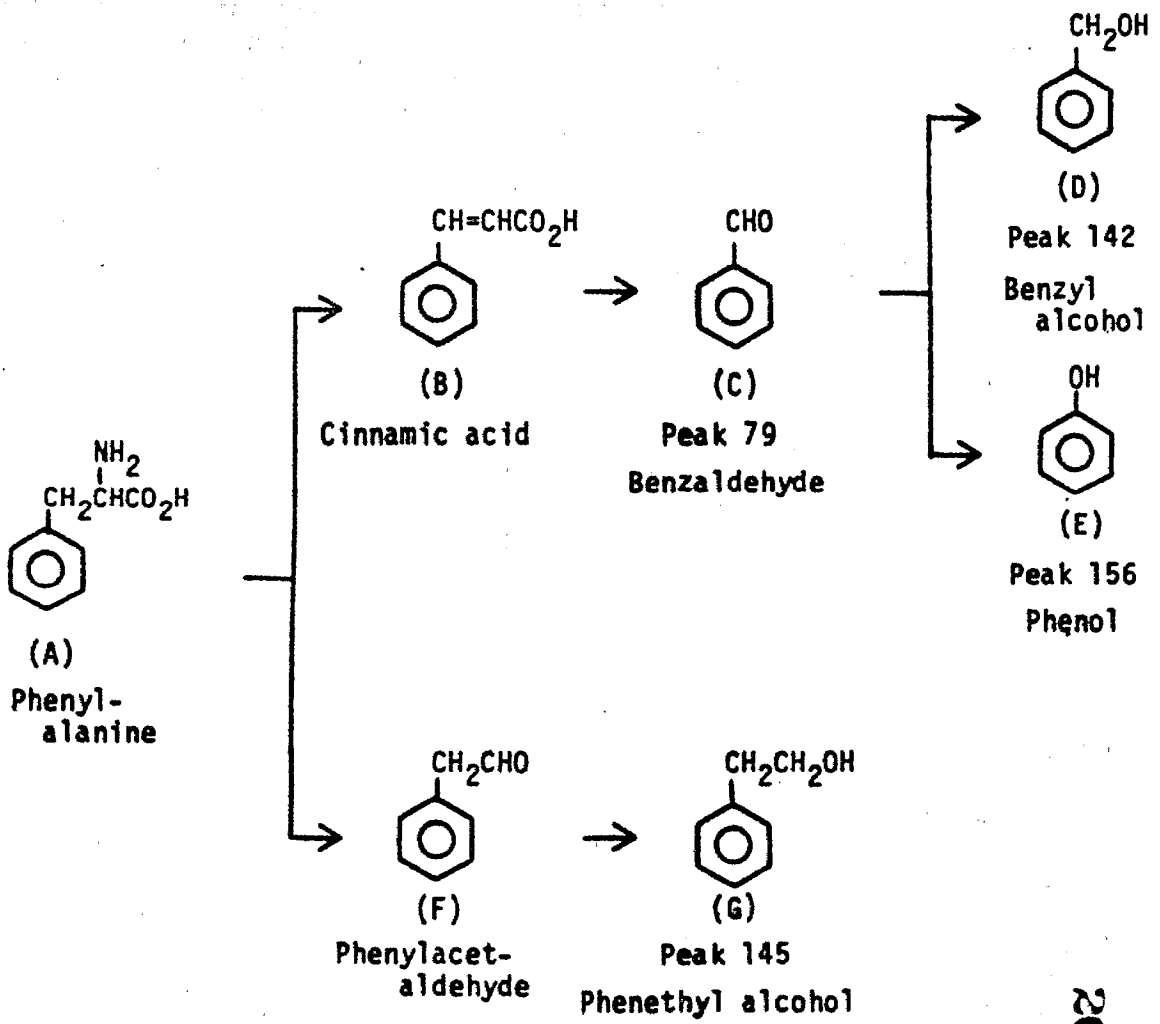

Figure 11.

2000587012

Source: https://www.industrydocuments.ucsf.edu/docs/xtyk0000

The degradation of chloroplast pigments begins with the onset of senescence. Chlorophyll has a long hydrophobic terpenoid side chain, consisting of the alcohol phytol esterified to a propionic acid substituent in ring IV. The chlorophylls are tetrapyrrole pigments in which the porphyrin ring is in the dihydro form and the central metal atom is magnesium. Chlorophyll is a diester of a dicarboxylic acid (chlorophyllin), one group esterified with methanol, the other with phytol alcohol.

Chlorophyll degradation results in the formation of phytol and methyl alcohol by splitting of the ester linkage. Further oxidation may lead to the formation of derivatives of the imide of maleic acid, with porphyrin compounds as intermediates. By thermal treatment and tissue dehydration during the curing process, chlorophylls decompose rapidly. Pyrrole is the basic unit from which the tetrapyrrole pigments are derived. Therefore, it might be possible that the pyrroles (Peak 160, 172, 192-2, and 206) identified in this study are the degradation compounds of chlorophylls. The color and quality of the flue-cured tobacco are affected by leaf maturity and the rates of decomposition of the pigments during curing.

Phytol (Peak 227) is released in the first step of chlorophyll degradation by breakage of the ester bond. The dehydration of the phytol alcohol results in the formation of several phytadiene isomers. Neophytadiene (Peak 149) is one of the isomers and is present in large quantity in the steam-distillate of flue-cured and burley tobacco. Neophytadiene increased during curing in this study.

Furans. Ten compounds with furan structure were determined in the volatile oils of this study. Furfural (Peak 65) imparts a sweet, yeasty, and buttery smoke flavor. The concentration of this compound increased from 0.5 µg/g at green sample to 3.2 µg/g at cured stage. 5-Methyl furfural (Peak 91) contributes sweetness and adds "body" to smoke. Furfuryl alcohol (Peak 103) has a bran-like odor, and adds oily flavor to smoke. The changes in the concentrations of 5-methyl furfural and furfuryl alcohol tended to increase during curing.

The compounds found in these profiles have structures that suggest their relationship to a larger molecule present in green tobacco. Generally the concentration of the degradation products of the many precursors increased during curing and many of the smaller degradation products degraded even further to smaller compounds. Sixty-eight of the 72 compounds identified and quantified in this study contained an oxygen in the structure of the molecule. This is quite indicative that oxygenation of many compounds contribute to the flavor and aroma of tobacco and tobacco smoke.

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GRADUATE STUDENTS:

Chang, K. W. (Korea), Ph.D., under W. W. Weeks and J. A. Weybrew

PAPERS PRESENTED AT PROFESSIONAL MEETINGS:

Campbell, C. R., and W. W. Weeks. Flavor constituents from close grown flue-cured tobacco. 37th Tobacco Chemists' Research Conference, Washington, D.C., October 1983.

Weeks, W. W., and H. Seltmann. Volatile and non-volatile compounds after different degrees of sucker control in flue-cured tobacco. 37th Tobacco Chemists' Research Conference, Washington, D.C., October 1983.

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Source: https://www.industrydocuments.ucsf.edu/docs/xtyk0000

ON-FARM TESTS 1983

North Carolina burley growers experienced a difficult season in 1983. Rainy weather caused a delay in seeding beds and transplanting was two weeks or more later than normal. Wet weather continued through transplanting followed by a sustained drought that lasted most of the growing season. Most counties had late rains in time to produce moderate to good yields but an early frost damaged about 50% of the crop. Curing weather was moderate to good in most counties. The quality of leaf on the three sale floors in the state was quite good. Producers that harvested early enough to escape frost injury had excellent quality.

All dollar values in this report are based on 1983 support prices.

A total of 34 tests were conducted in twelve counties. Data were not collected from some tests because of irregular growth caused by drought stress or because of frost injury.

Replicated Varieties 1983

Data are presented from six locations. A seventh test was severely injured by frost before harvest and is not included. Tobacco at most locations was under stress for much of the growing season. Variation in yield within and among locations was high. Overall test yields ranged from a low of 2300 pounds per acre at two locations to a high of 3500 pounds at one location. The other three tests were in the 28 to 2900 pound range. No single variety or hybrid was a consistent top yielder at all test sites. Surprisingly, the overall lowest yielder, Burley 64, was tops in Mitchell County at 3200 pounds.

The highest yielding varieties in this test are normally Kentucky 14, Kentucky 15, Kentucky 10, and the hybrids 14 x L8, 21 x 10, and 21 x 14. The blackshank resistant varieties are normally lower yielding with Virginia 509 usually highest. Kentucky 17, Virginia 528, and Burley 64 are in the moderate to low yielding group. Any of the varieties or hybrids included in these tests will produce adequate yields with reasonable cultural practices. Differences in yields among varieties are much less in some seasons. Many producers tend to place too much emphasis on yield per acre when this factor is of much less importance under a poundage quota than it was under an acreage allotment program. Disease resistance should be the first factor considered in selecting a variety.

Quality, as measured by selling price, has been similar for all varieties over the past several years. Minor variations in price will occur in tests of this type as a result of differences in curing locations within a barn or as a result of other minor variations that occur in conducting a field trial. At the higher elevations in the mountains of western North Carolina, early transplanting will consistently result in better curing conditions and thus higher quality tobacco. Crops

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transplanted as soon as possible after May 20 can be harvested early enough for the cure to progress through the yellowing stage before really cool weather. Crops transplanted later than about June 10 are subjected to serious risk during curing. Green color can be set in the leaf if temperatures drop below the 50's if the cure has not progressed through the yellowing stage.

Replicated Varieties 1983
Averages for 6 Tests

Variety	Acre Yield	$/cwt	$/Acre
1. Ky 14	2965	181.76	5393
2. Ky 15	2746	177.45	4882
3. Ky 10	2798	182.10	5116
4. Bu 64	2566	180.44	4650
5. Ky 17	2685	180.08	4856
6. Va 509	2746	178.65	4917
7. 14 x L8	3061	179.96	5508
8. 21 x 10	3098	181.12	5610
9. Va 528	2664	181.03	4833
10. 21 x 14	3012	182.13	5492

Sucker Control 1983

Tobacco at most locations was drought stressed at the time chemicals were applied. Growth was uneven and some tests were not carried to completion where irregular growth was extreme. Rains came to most of the area after chemicals were applied resulting in moderately heavy sucker pressure and satisfactory yields.

Sucker control chemicals appear to be less effective when applied to drought stressed plants. Systemics are absorbed more slowly and not as completely and proper coverage with good run-down is more difficult with contacts. Adequate control, however, can still be obtained as indicated by results obtained in this series of tests. These treatments were applied with a 3-nozzle hand-held boom pressurized with CO_2 gas and designed to simulate results obtained by a regular multi-row boom. Careful use of a single nozzle hand sprayer will give equal, and in some cases, even better results. A major portion of North Carolina burley is still treated with hand sprayers.

The percent sucker control obtained was good to excellent with all chemicals used. The poorest, but still adequate, results were obtained with two applications of contact in Treatment 3. The best use of contacts are obtained when they are applied before topping, in the button stage, followed by topping and the application of another spray containing maleic hydrazide as in Treatment 7, or FST-7 as in Treatment 5. Prime + may also be used as a sequential application. The sequential

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application requires two sprayings and more expense for the extra chemical. Producers who use the sequential system feel that the advantages outweigh the disadvantages. The contact can be applied in the button stage before any suckers begin to grow. The primary sucker buds, which seem to be the most vigorous suckers the plant produces, are killed outright. The secondary and tertiary suckers are then easier to control. Maleic hydrazide or FST-7 will result in complete sucker control that lasts longer than 35 days when applied after a contact. Topping can be on a more flexible schedule, or anytime after application of the contact until the second spray is applied which can be 3-10 days later (usually about a week). There are no suckers to pull at topping. The sequential control program is currently the closest method available for total control of both pre- and post-topping suckers.

A single application of maleic hydrazide applied after topping is presently the most popular treatment. In the North Carolina area, control will last from 21 to 28 days depending on the amount of sucker pressure. Some producers tend to top a little later than normal or harvest a little earlier in order to harvest sucker-free tobacco.

FST-7 is also a good sucker control chemical. Control from a single application will last a little longer than a single application of maleic hydrazide.

A single application of Prime + applied after topping will give season long sucker control. However, complete coverage is difficult to obtain. The overall average percent control reported here was 86% for 7 tests. Five of the 7 tests resulted in control of 90% or higher with 3 of 97% and 1 of 98%. The Chambers location in Haywood County resulted in only 36% control. The degree of drought stress was extreme at time of application.

The maleic hydrazide is applied to the leaves. It can be applied in 20 to 60 gallons of water per acre. The other 3 types of chemicals, contacts, FST-7, and Prime + require about 50 gallons of water per acre. The spray is directed downward toward the top of the plant and must be in sufficient volume to run down the stalk and wet each sucker bud. First time users frequently tend to go too fast for best results.

For consistent results with sucker control chemicals, growers should (1) read the label and follow directions, (2) measure chemicals accurately, and (3) calibrate the sprayer.

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Sucker Control 1983
Averages for 7 Tests

Treatment	% Cont	Suck. No/Pl	Gn Wt gms/Pl	Acre Yield	$/cwt	$/Acre
1. Topped Not Suckered	--	5.6	574	2542	179.97	4571
2. Hand Suckered	49	10.3	292	2781	180.44	5019
3. OST Button + OST Topping 1.5 GPA	77	2.1	133	2954	178.12	5262
4. KMH 1.5 GPA	93	1.3	43	3195	178.89	5705
5. Contact @ 1.5 GPA + FST-7 @ 8 qts	97	.3	18	3142	179.75	5648
6. Prime + @ 1 GPA	86	.6	82	3056	178.27	5433
7. Contact @ 1.5 GPA + KMH @ 1.5 GPA	99	.4	6	3304	180.60	5957
8. FST-7 @ 9 qts	97	.3	15	3024	178.10	5395

Curing Tests

Supplemental heating systems for curing burley are designed, primarily, for use during inclement weather. Curing damage usually does not occur until the cure has progressed through the yellowing stage and parts of the leaf begin to die. Serious damage can begin when damp weather prevails for longer than a 24 hour period. Damp conditions at night are not serious if the barn is ventilated well enough to dry out the following day. Houseburn damage is measured in both weight loss and lowered quality.

Supplemental heat is used when damp weather has lasted for a 24 hour period and it appears that conditions are going to persist. Heaters raise barn temperatures by 6-8 degrees fahrenheit or a little higher and result in reduced humidity within. Higher temperatures may set undesirable colors in the leaf.

Favorable curing conditions prevailed at both locations in 1983 and supplemental heat was used very little. Consequently, no substantial differences in weight or quality occurred between treatments receiving supplemental heat and those that were totally air cured.

Curing Test 1983
Averages for 2 Tests

	Acre Yield	$/cwt	$/Acre
With Heat	3203	178.81	5647
No Heat	3173	177.14	5524

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EXPLANATION OF SIZE DISTRIBUTION OF FARMS:
ECONOMIC MODELS AND EMPIRICAL APPLICATIONS NC 3835

Daniel A. Sumner, Project Leader

I. Summary of Research:

The tobacco part of this project will focus on developing a model explaining the current distribution and changes in the size distribution of tobacco farms and tobacco enterprises. The impact of policy, prices, interest rates and characteristics of farmers will all be considered.[1]

We derive a model of farm size from the basic theory of the firm. This yields implications for the density function for a population or potential population of farms. Different common measures of size are shown to react differently to changes in underlying determinants. Farmer human capital relates strongly to farm size in tobacco as in other industries.

The empirical implementation of the model to tobacco has initially examined the impact of the tobacco program on size. We find little direct influence of the program on size except through the restrictions on geographic movement of the industry. Tobacco quota income would fall and there would be a large expansion of the total industry.

II. Graduate Students: Bennett Bradley, RJR undergraduate research apprentice.

III.

IV. Publications:

V. Manuscripts Accepted for Publication:

"The Effects of Elimination of Price Supports and Supply Restrictions for Tobacco," forthcoming 1984, National Planning Association, with Julian M. Alston.

Deregulation of the U.S. Tobacco Industry: An Economic Analysis of the Effects of Removal of Price Supports and Supply Controls. Accepted for publication as a monograph by The American Enterprise Institute for Public Policy Research, 1984, with Julian M. Alston.

"The Tobacco Program: Where Would You Be Without It." The Flue Cured Tobacco Farmer, March 1984, pp. 34-38, with Julian M. Alston.

VI. Manuscripts in Review:

"On Explaining Farm Size Distributions," Working Paper No. 28, December 1982, revised draft under review for publication, with James Cochell.

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Source: https://www.industrydocuments.ucsf.edu/docs/xtyk0000

"Consequences of Deregulation in the U.S. Tobacco Industry," under review for presentation at the 1984 annual meeting of The American Agricultural Economics Association, with Julian M. Alston.

"Determinants of Size of Dairy Farms: The Role of Human Capital," under review for presentation at the 1984 annual meeting of The American Agricultural Economics Association, with James Leiby. The model and procedures are developed to be applied to our tobacco size research but are used to discuss dairy farm data in this paper.

VII. Papers Presented at Professional Meetings:

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Source: https://www.industrydocuments.ucsf.edu/docs/xtyk0000

CIGARETTE SMUGGLING: THE DEMAND TO SMUGGLE AND THE DEMAND TO CONSUME NC3710

Daniel A. Sumner, Project Leader

I. Summary of Research:

We have developed econometric and simulation models of the impact of taxation and other factors on the demand for cigarettes and tobacco. Collection of detailed time series/cross section data and putting the data in a form useful for computer analysis has been accomplished. This unique information set is now available to researchers interested in any aspects of the demand for the major tobacco product (cigarettes). This data base includes quantities, prices, taxes, demographic factors and regulations.

A simulation model of the impact of cigarette tax policy was developed and applied to study the 1983 tax change. We were successful in predicting the major first round consequences of the 1983 federal tax increase. This model has also led to research applications relating to the other commodities.

A model was developed to explain the implications of removal of supply restrictions and price supports for U.S. tobacco. Using current data and parameter estimates we find that an end of the "Tobacco Program" would entail a slight fall in the price of cigarettes and a small rise in the quantity demanded. The quantity of tobacco demanded for exports and import replacement would expand significantly.

II. Students: Bennett Bradley, RJR undergraduate research apprentice.

III.

IV. Publications:

"An Economic Analysis of the 1983 Increase in the Federal Excise Tax on Cigarettes," Economics Research Report No. 44, North Carolina State University, April 1983, with Michael Wohlgenant.

"The 1983 Federal Cigarette Tax Increase," Tar Heel Economist, August 1983.

"Effects of Cigarette Tax Changes," in Charles R. Pugh (ed.) Tobacco Issues for the '80s, proceedings of a Conference, March 8-9, 1983, School of Agriculture and Life Sciences, North Carolina State University, 1983.

"Cigarette Taxes, Prices and Demand," Tar Heel Economist, August 1983 (by W. Bennett Bradley).

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Source: https://www.industrydocuments.ucsf.edu/docs/xtyk0000

V. Manuscript Accepted for Publication:

"Consequences of Elimination of the Tobacco Program," N.C. Agricultural Research Bulletin, March, 1984, with Julian M. Alston.

VI. Manuscripts in Review:

"Effects of an Increase in the Federal Excise Tax on Cigarettes," Faculty Working Paper No. 26, October 1982, under review for publication with Michael Wohlgenant.

VII. Papers Presented at Professional Meetings:

"Effects of an Increase in the Federal Excise Tax on Cigarettes," with Michael Wohlgenant, annual meetings of The American Agricultural Economics Association, August 1983. Abstract in the AJAE, December 1983.

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Source: https://www.industrydocuments.ucsf.edu/docs/xtyk0000

Analysis of International
Trade Restrictions
NCARS No 2105
Project Leader Paul R. Johnson

1. Previous work on international trade in tobacco has led to a book length manuscript on both domestic and international dimensions of U.S. and foreign tobacco policy.

   Work specific to the past year has been to examine the reasons for the decline in demand for U.S. tobacco. Several forces at once are at work; exchange rate problems, worldwide recession, a competing supplier, and technologic change.

IV. Johnson, P.R. and D. Norton, 1983
"The Social Cost of the Tobacco Program Redux."
Am. Journ. of Agric. Econ. 65(1)

V. The Economics of the Tobacco Industry.
Praeger Special Studies, N.Y. Spring 1984

VI. Johnson, P.R. "World Demand for Tobacco"
"The Role of Prices and Exchange Rates."
Paper presented at a conference, Tobacco Issues for the '80's, March 8-9, 1983.

2000587023

Source: https://www.industrydocuments.ucsf.edu/docs/xtyk0000

Title: NC 3671: Tobacco Insect Control

Project Leader: Emmett P. Lampert

I. Summary of Research:

A. Evaluation of foliar application of various insecticides for control of tobacco insect pests.

Three experiments were conducted at the Central Crops Research Station to evaluate the efficacy of candidate insecticides on the insect pest of flue-cured tobacco. All experiments were set up in a randomized complete-block design with three blocks. Plots were four rows (45" on center) 80 feet in length (approx. 45 plants). Treatments were applied using a John Deere Hy-Cycle 600 sprayer traveling at approximately 1.4 mph fitted with full cone nozzles. At 60 psi this spraying set up delivered approximately 25 gallons of finished spray per acre. Evaluations were made for tobacco hornworms, tobacco budworms, green peach aphids and flea beetles. Materials tested not only included chemical insecticides but also insect growth regulators, various formulations of Bacillus thuringiensis and B. thuringiensis extotoxins. The experiments were set up in test containing 9, 8 and 7 treatments and all were repeated twice during the season. Because of this complexity and bulk, a reader is referred to the 1983 "Chemical test in Flue-Cured Tobacco in 1983" for the results of specific material against specific insects.

In summary, relatively low numbers of green peach aphids were present this season. Because of these low densities, little information on the efficacy against the green peach aphid could be obtained. Tobacco budworms appeared early this season at fairly significant levels and were very difficult to control. Presumably due to the weather which kept the plant bud tight and prevented the larvae for becoming exposed to the insecticides. Of the materials tested, only FCR-1272 provided significantly better budworm control than the standards (Sevin and Orthene). This was the most promising new budworm product evaluated.

During the second replication of the experiments, significant numbers of tobacco hornworms were present and could be evaluated for efficacy. Almost all of the materials provided significant control as compared to the check, but most were not significantly different from the standards.

B. Effects of insecticides, fungicides, and sucker control chemicals on survival of the green peach aphid.

The green peach aphid, Myzus persicae (Sulzer), has been the major insect problem for North Carolina tobacco farmers in most of the past several years (Southern 1984). The purpose of this study was to determine the effects of the insecticides, fungicides, and sucker control chemicals most widely used on tobacco in North Carolina on survival of the green peach aphid.

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Treatment solutions were prepared by placing 0.5 g AI of the pesticide containing 25 ml water in a Petri dish. Twenty alate aphids were placed on their backs on doublestick tape stuck to a microscope slide (four replications of 20 aphids each per chemical). The microscope slides were immersed in the chemical solution for 30 sec and then drained vertically for 15 min. Then, each microscope slide was placed in a Petri dish and kept at 16:8 LD and at room temperature. After 24 h; survival of the aphids was determined by observing leg movement after stimulation.

In this preliminary study, the use of Azodrin, Orthene, Thiodan, Diazinon, Lannate, Ridomil, Prime +, and Offshoot-T resulted in 100% mortality of the green peach aphid (Table 1). The effects of the chemicals on survival of the green peach aphid will also be determined in greenhouse and field studies. The results of these studies may help a grower in deciding which chemical to use when faced with more than one control problem.

Reference

Southern, S. 1984. Insect Management. In: 1984 Tobacco Information, North Carolina Agricultural Extension Service. pp. 84-99.

Table 1. Green peach aphid control in the laboratory

Treatment	Mean % Survival[a/]	Standard Deviation
Insecticides		
Azodrin (monocrotophos)	0	0
Orthene (acephate)	0	0
Thiodan (endosulfan)	0	0
Diazinon	0	0
Lannate (methomyl)	0	0
Dipel (*Bacillus thuringiensis*)	100	0
Sevin (carbaryl)	54	10
Fungicides		
Manzate (mancozeb)	91	10
Agrimyein (streptomycin sulfate)	96	8
Ridomil (metalaxyl)	0	0
Benlate (benomyl)	1	3
Sucker Control Chemicals		
Royal MH-30	23	10
Prime +	0	0
Offshoot-T	0	0
Control (water)	98	3

[a/] Based on 4 replications of 20 aphids per treatment.

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C. Evaluation of the role of alate aphids in transmission of potato virus Y (PVY) and substrate formation of sooty mold.

Studies were conducted in a commercial field of flue-cured tobacco in Cartaut Co. to examine the role of aphids in the transmission of PVY. Aphid activity was monitored in 4 test plots through the use of flight interception traps and alighting traps. The interception traps were maintained at 6 feet above the soil surface and the alighting traps were kept at the top of the canopy. In the center of each plot, a varing number of PVY infected potato plants served as the inoculum source. All aphid traps were changed on a daily basis and the tobacco surrounding the potatoes was sampled twice per week for presence of PVY. All aphids were cleared and mounted on slides and sent to Dr. C. F. Smith for genus and species determination.

The results of the aphid catches from the interception traps and the alighting traps are summarized in Figures 1 and 2, respectively. It is interesting to note the differences in seasonal abundance the two trapping techniques represent. It appears as though the traps are trapping two distinct aphid behaviors. The interception traps the aphids that are flying above the plant canopy and may or may not be landing while the alighting trap collects only those aphids which are landing.

Sixteen species were collected which were considered abundant (comprised at least 3% of total season's catch) and of these many are potential vectors.

The results of the tobacco plant surveys for PVY are summarized in Figure 3. As was expected, the plots with the highest level of source inoculum had the highest level of PVY at the end of the season. Looking at Figures 2 and 3 there is little similarity between the aphid activity and the amount of PVY in any of the plots. However, if we look at the proportion of aphids alighting in tobacco that are green peach aphids (Figure 4) a striking similarity is observed.

Early in the season, before Julian date 160, a low level of PVY was found in the potatoes. We believe this was due to both transient aphids and the green peach aphid. However, since the level of PVY increased significantly about 10 days after the green peach aphid became abundant, we believe the green peach aphid is primarily responsible for the secondary spread of PVY within the tobacco crop.

Field experiments were conducted at the Central Crops Research Station to determine the effects of green peach aphids on the yield and quality of tobacco. A split-plot design with 4 blocks was set up to examine the effects of various levels of aphid control split with fungicide application for control of sooty mold on the yield and quality of the test tobacco. A significant difference in quality, as measured by the quality index, was observed in the third harvest. However, due to the overall low quality of the tobacco these results are inconclusive. Further experiments are being conducted in the laboratory to answer additional questions of honeydew production rates and honeydew suitability for sooty mold growth.

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D. Continued development of improved sampling methods for insect abundance in tobacco.

During the 1983 tobacco growing season, data were collected on a weekly basis from two tobacco fields at the Central Crops Research Station. Random samples of 15 or 20 single plants were collected and examined for insects. All insects present (both pests and beneficials) were counted and recorded. Data were also collected on plant height, leaves per plant and leaf area.

Tobacco budworms, flea beetles and green peach aphids were fairly abundant and provided useful information. The tobacco hornworm, however, was present at relatively low levels. Coccinelid adults and larvae, stilt bugs and syrphid larvae were all quite abundant and provided useful information on beneficial insects.

Using this data, relationships between mean and variance per plant were determined for all the above insects except the tobacco hornworm. These relationships made it possible to determine the number of plants that are necessary to sample for each insect to estimate their density with a predetermined level of precision.

Aphid data were further analyzed to determine the distribution of aphids with the tobacco plant throughout the season. The highest densities of aphids were found from 3 to 5 leaves below the terminal growing point. This information is useful to allow subsampling of a plant and determination of total plant density.

E. Test and demonstration of insect management practices for burley tobacco: insect abundance and phenology.

During the 1983 growing season, test plots were established in commercial plantings of burley tobacco in Haywood, Madison and Yancey counties. These plots were designed to evaluate the economics of various insect management programs. Information was also collected from these plots on insect abundance and phenology, which will be reported on here.

To determine the abundance of insects, weekly examinations of 5 consecutive plants from 2 rows in the untreated plots were made and all insects recorded. Counts were made on insect pests (green peach aphid, tobacco hornworm, tobacco budworm and flea beetle) as well as beneficial insects (Campoletis, syrphid and coccinelid adults and larvae). Of these, only the flea beetles were abundant enough to warrant presentation here.

Flea beetles were most abundant in Yancey Co., followed by Madison and Haywood counties with peak densities of 49.0, 22.8 and 15.4 flea beetles/plant, respectively (Figure 5).

All three counties exhibited peaks of adult densities approximately 40 and 75 days after transplanting. These correspond to the first and second generation adults. In Madison and Yancy counties, a slight peak was noticed

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shortly after transplanting. This corresponds to the colonization of the field by overwintering adults. This information is being used to determine optimum sample sizes for density estimation and to develop control measures based upon phenology.

II. Graduate Students:

Committee Chairman - 2 (M.S.)
Committee Member - 3 (Ph.D.)

III. Postdoctoral Fellows:

Dr. James E. Throne - R. J. Reynold Fellowship

IV. Publications:

Lampert, E. P., D. L. Haynes, A. J. Sawyer, D. P. Jokinen, S. G. Wellso, R. L. Gallun and J. J. Roberts. 1983. The effects of regional releases of resistant wheats on the population dynamics of the cereal leaf beetle (Coleoptera: Chrysomelidae). Ann. Entomol. Soc. Amer. 76:972-980.

V. Manuscripts Accepted for Publication:

Lampert, E. P. and P. S. Southern. 1984. Control of an uncommon pest attacking flue-cured tobacco-1982. Insecticide and Acaricide Tests. Vol. 9.

Lampert, E. P. 1984. Control of tobacco budworms and tobacco hornworms with foliar insecticides-1982. Insecticide and Acaricide Tests, Vol. 9.

Lampert, E. P. 1984. Control of tobacco budworm and tobacco hornworm with Bacillus thuringiensis var. Kurstaki-1982. Insecticide and Acaricide Tests, Vol. 9.

Lampert, E. P. 1984. Control of tobacco budworms and tobacco hornworms with foliar insecticides-1983. Insecticide and Acaricide Tests, Vol. 9.

Lampert, E. P. and D. L. Haynes. 1984. Temporal and spatial changes in abundance of the asparagus miner, Ophiomyia simplex (Loew) (Diptera: Agromyzidae), in Michigan. Environ. Entomol.

VI. Manuscripts in Review:

Margolies, D. C., E. P. Lampert and G. G. Kennedy. A sampling program for the twospotted spider mite, Tetranychus urticae Koch (Acari: Tetranychidae) in Peanut. Submitted to J. Econ. Entomol. 1/17/84.

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Source: https://www.industrydocuments.ucsf.edu/docs/xtyk0000

Lampert, E. P. and D. L. Haynes. Population dynamics of the cereal leaf beetle, Oulema melanopus (Coleoptera: Chrysomelidae), at low population densities. Submitted to Environ. Entomol. 2/14/84.

VII. Papers Presented at Professional Meetings:

Lampert, E. P. 1983. Seasonal distribution of the green peach aphid on flue-cured tobacco. 1983. National E.S.A. Meeting, Detroit, MI, Nov. 29.

Gray, S. M. and E. P. Lampert. 1983. Primary spread of potato virus Y (PVY) as related to aphid and inoculum dynamics. National E.S.A. Meeting, Detroit, MI, Nov. 29.

IX. Acknowledgements:

I would like to thank Mr. George B. Clark, former project technician for keeping the day-to-day operations of the program running smoothly. I would also like to acknowledge Mr. Alan S. Stephenson, the current project technician, for picking up where George left off. I would also like to thank Mr. Stewart Gray and Dr. James Throne for their assistance in the research program. The cooperation provided by Mr. Wallace Baker and Mr. Alton "Bo" Wood (Superintendent and Tobacco Supervisor at the Central Crops Research Station, respectively) has greatly facilitated this research and last but not least, the editorial and secretarial assistance of Ms. Connie Satterwhite is gratefully acknowledged.

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Source: https://www.industrydocuments.ucsf.edu/docs/xtyk0000

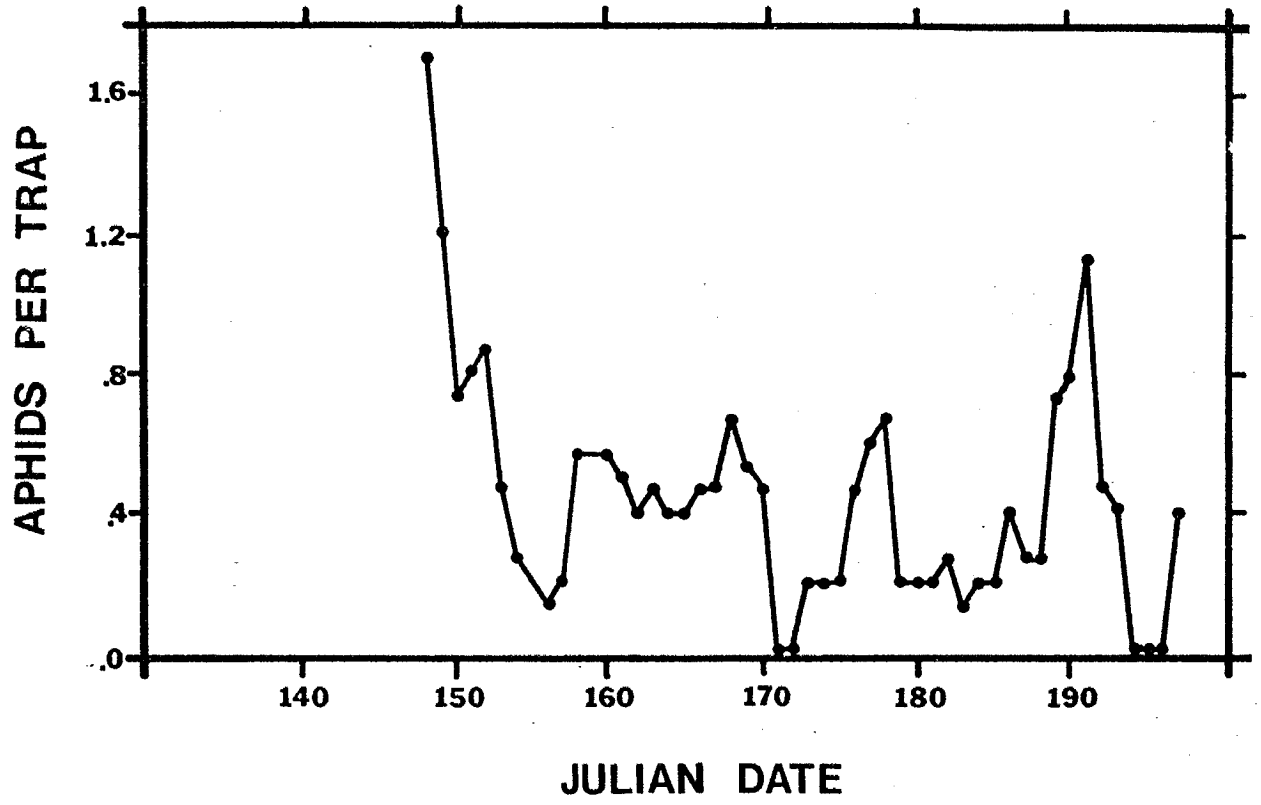

Figure 1. Mean aphids per trap collected in the interception traps in Carteret County in 1983.

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Source: https://www.industrydocuments.ucsf.edu/docs/xtyk0000

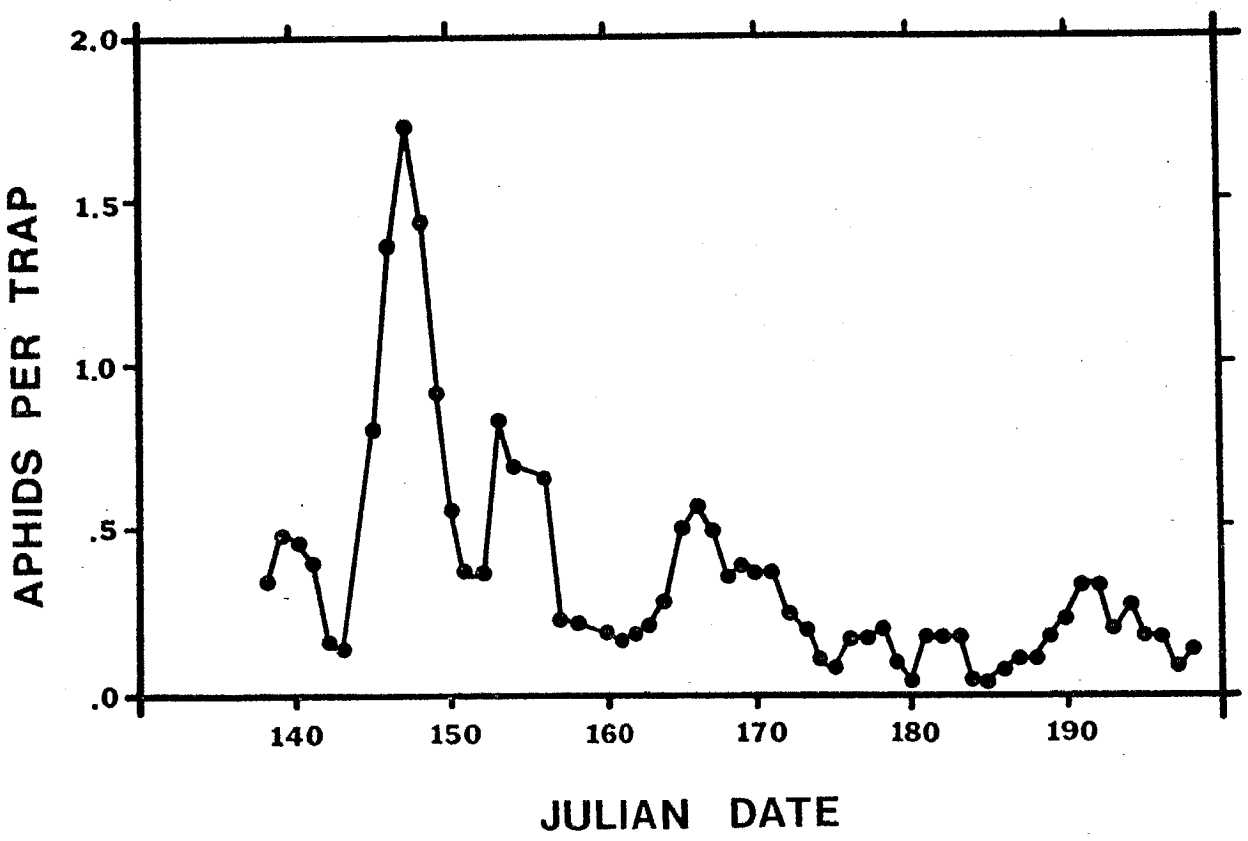

Figure 2. Mean aphids per trap collected in the alighting traps in Carteret County in 1983.

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Source: https://www.industrydocuments.ucsf.edu/docs/xtyk0000

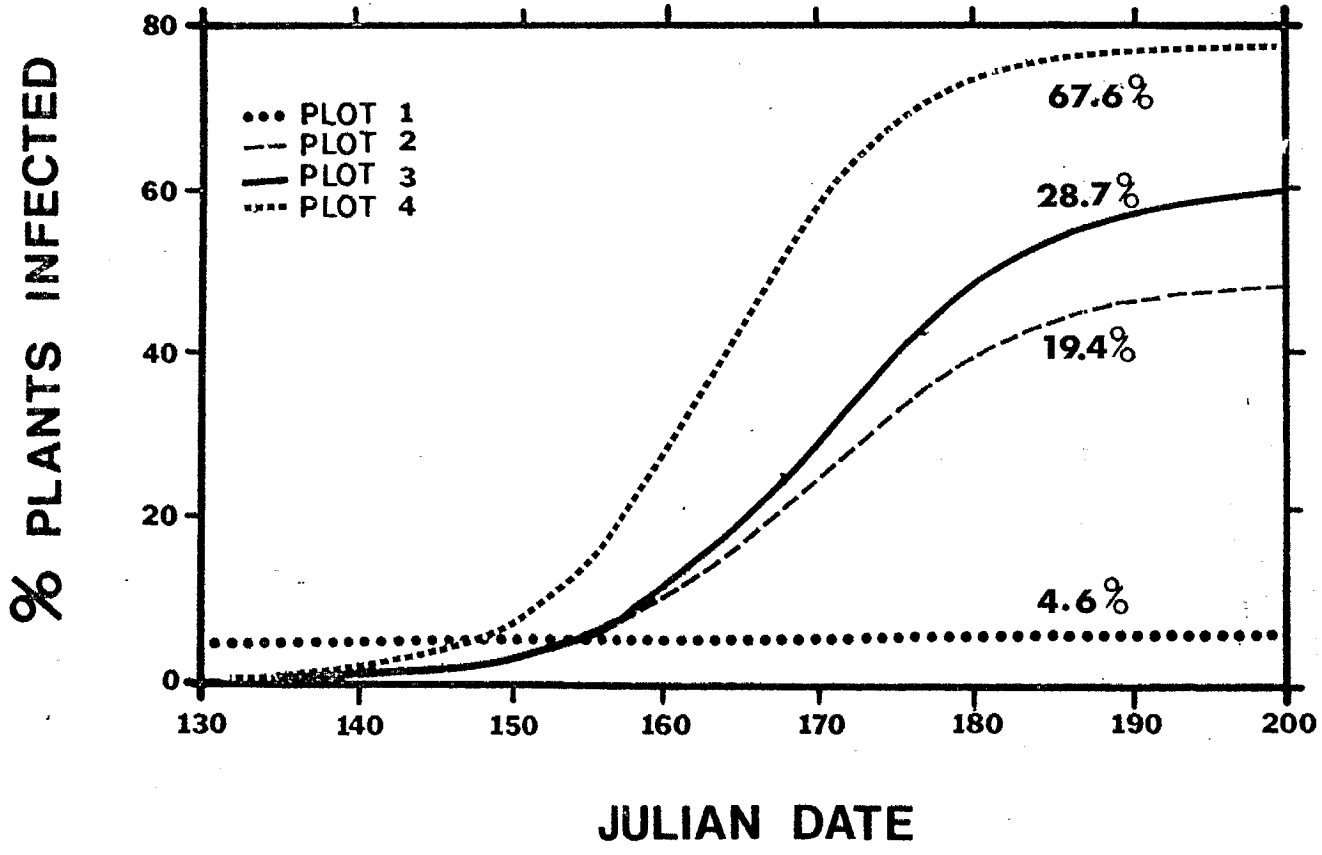

Figure 3. Disease progress curves for the four plots designed to monitor the spread of viruses from a localized source. Numbers near lines represent the final inoculum level in the source plants. Data collected in 1983 in Carteret County.

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Source: https://www.industrydocuments.ucsf.edu/docs/xtyk0000

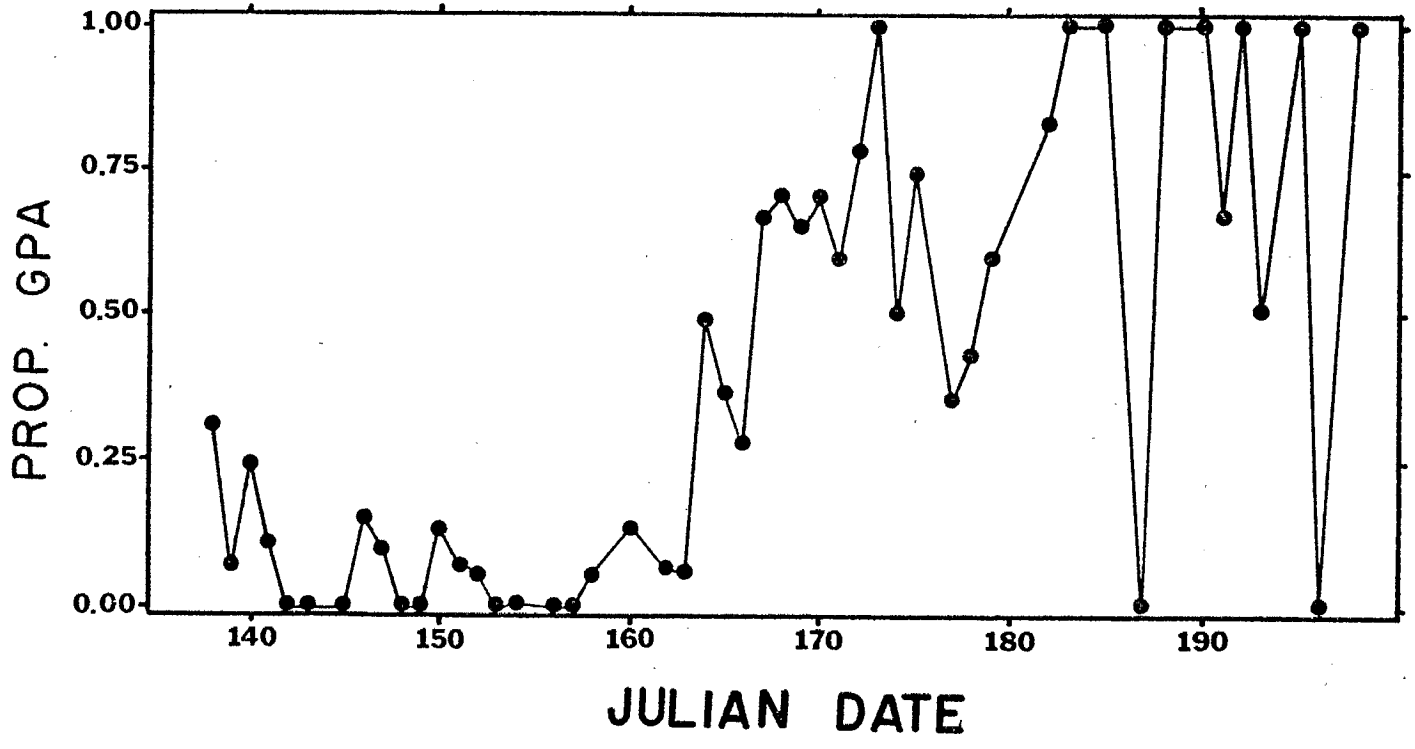

Figure 4. Proportion of tne total catch in the alighting traps that was the green peach aphid.. Traps operated in Carteret County in 1983.

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Source: https://www.industrydocuments.ucsf.edu/docs/xtyk0000

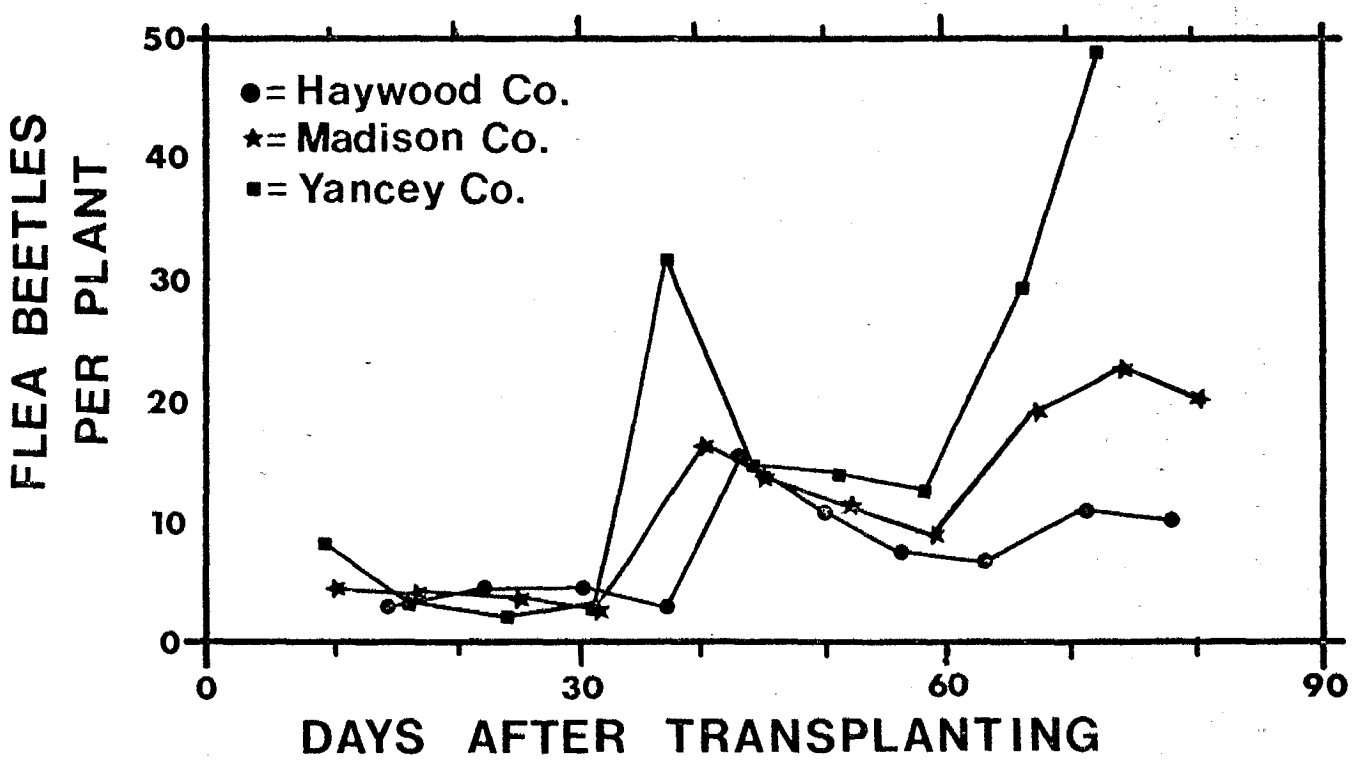

Figure 5. Mean number of flea beetle adults per plant versus days after transplanting for the three counties surveyed in 1983.

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Source: https://www.industrydocuments.ucsf.edu/docs/xtyk0000

TOBACCO INSECT CONTROL MEANS TO IMPROVE TOBACCO SAFETY
USDA, ARS-NRP 20882

D. Michael Jackson, Research Entomologist

I. Summary of Research:

A. Control of Insects on Flue-Cured Tobacco with Bacillus thuringiensis var. kurstaki Spray Formulations:

One aqueous and two wettable powder formulations of Bacillus thuringiensis var. kurstaki were tested for control of artificial infestations of tobacco budworms, Heliothis virescens (F), and tobacco hornworms, Manduca sexta L., on flue-cured tobacco in 3 separate tests at Oxford, NC. Tobacco was transplanted on May 18 into a field of Vance sandy loam soil which had been treated with Paarlan (2.3 l/ha) and Ridomil (2.3 l/ha) on May 4. Plants were placed on 55.9-cm centers with 1.2 m between rows. After experiments 1 and 2, the field was cutoff just above the ground, and two weeks later one sucker per plant was retained to form new plants used in experiment 3. Plants in experiments 1 and 3 were infested with one 2- to 5-day-old tobacco budworm larva from a laboratory colony. Plants in experiments 2 and 3 were infested with two 3 to 4-day-old tobacco hornworm larvae also from a laboratory colony. For each test, 5 replications of 15 or 25-plant plots were set up in a randomized complete block design. Plots were treated with a CO_2-powered backpack sprayed with an adjustable nozzle at a rate of 233.8 l/ha with 4.2 kg/cm^2. Plants were examined for insects 4 and 7 days after spraying. The weather was hot and dry for at least 2 days after each experiment.

Both the aqueous formulation (ABG-6122) and the 2X wettable powder (ABG-6139) at 2 rates were equally effective against hornworms and budworms as Dipel WP at 1.12 kg/ha, and equal or superior to Dipel WP at 0.56 kg/ha (Table 1). These treatments were also equal in efficacy to Orthene except at 4 days in experiment 1. Significantly fewer pest larvae survived in any of the spray treatments than in the check plots. Test materials were provided by Abbott Laboratories, North Chicago, Illinois.

B. Insect and Sucker Control with Tank Mix Combinations of Four Insecticides and Two Sucker Control Chemicals on Flue-Cured Tobacco:

Tank mixes of two sucker control chemicals and four insecticides were tested for control of tobacco budworms, tobacco hornworms, and tobacco suckers. Efficacies of the insecticides were evaluated in a field planted to 'Speight G-70' on May 18. On May 4 this field was treated with Paarlan (2.3 l/ha) and Ridomil (2.3 l/ha). Three applications of 2% (July 15) and 4% (July 18 and 21) Off-Shoot-T were applied. Two days prior to treatment spraying, two 3-day-old laboratory-reared tobacco budworm larvae and two 5-day-old laboratory-reared tobacco hornworm larvae were placed on the upper portion of each plant. Treatments were replicated four times in plots of 30 plants each in a randomized complete block design. Treatment spraying was done on July 29 using a CO_2-powered backpack sprayer at 467.7 l/ha at 2.8 kg/cm^2. Surviving insects were counted 3 days after spraying. Sucker control and leaf burn damage were evaluated in a test done on 'Speight G-28' tobacco that was

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Table 1. Tobacco budworm and hornworm control using 3 formulations of Bacillus thuringiensis on flue-cured tobacco, Oxford, NC, 1983.

Treatment and Rate ai/ha	Experiment 1			Experiment 2		Experiment 3	
	Tobacco budworm larvae/25 plants		Average[1] budworm damage rating	Tobacco hornworm larvae/15 plants		Tobacco budworm larvae/25 plants	Tobacco hornworm larvae/25 plants
	Days post-treatment			Days post-treatment		Days post-treatment	
	4	7		4	7	4	4
ABG-6122 aqueous 1.17 l	5.4 bc[2]	3.0 abc	1.46 b	0.0 a	0.0 a	1.6 ab	0.0 a
ABG-6122 aqueous 2.34 l	4.6 b	1.8 a	1.54 b	0.0 a	0.2 a	1.6 ab	0.0 a
ABG-6139 WP 0.28 kg	5.6 bc	2.8 abc	1.64 b	0.2 a	0.0 a	1.0 ab	0.0 a
ABG-6139 WP 0.56 kg	4.0 b	1.2 ab	1.53 b	0.0 a	0.4 a	1.4 ab	0.2 a
Dipel WP 0.56 kg	7.8 c	4.0 bc	1.66 b	0.0 a	0.0 a	3.8 b	0.0 a
Dipel WP 1.12 kg	4.6 b	1.6 ab	1.52 b	0.0 a	0.0 a	1.2 ab	0.0 a
Orthene 75 SP 1.12 kg	0.6 a	0.6 a	1.20 a	0.0 a	0.0 a	0.6 a	0.0 a
Check	13.8 d	4.8 cd	2.42 c	10.8 b	7.8 b	8.0 c	8.4 b

[1] Plants rated 0 (no damage) to 7 (plants topped) on day 7 after spraying.

[2] Means in the same column followed by the same letters are not significantly different (P=0.05) DMRT.

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transplanted on June 6. Before planting, this field was treated with Tillam (6.3 l/ha), Ridomil (2.3 l/ha), and Mocap Plus (9.4 l/ha). After transplanting, single applications of Enide (2.5 kg/ha), Ridomil (2.3 l/ha), and 4% Off-Shoot-T (467.7 l/ha) and three applications of Dipel WP (1.12 kg/ha) were made. Treatment sprays were applied on August 12 in the same manner as previously described. Three days, one week, and 5 weeks after treatment spraying, plants were examined for leaf burn and deformities. Plants were harvested in a normal fashion 4 times with the final priming on September 29. On October 4 all suckers were counted and weighed.

Tank mixing 4 insecticides with 2 sucker control chemicals did not alter insecticide efficacy against tobacco hornworms (Table 2). Tobacco budworm establishment was poor due to the large size of the plants and since Off-Shoot-T killed many leaves in the buds. Therefore, little information was gained on budworm control. All leaf burn recorded in these tests could be attributed to Off-Shoot-T, and not to any of the treatment combinations. All Royal MH treatments significantly reduced the number and weight of suckers, but the Bud Nip treatments were not better than the check plots treated with Off-Shoot-T. The addition of the 4 insecticides to the sucker control chemicals did not affect sucker control.

Table 2. Tobacco budworm, hornworm, and sucker control on flue-cured tobacco using tank mix combinations of insecticides and sucker control chemicals, Oxford, NC, 1983.

Treatment + rate ai/ha[1]		Larvae/30 Plants 3 Days After Spraying		Est. Suckers/ha 7 Weeks Post-Tmt	
		Budworms	Hornworms	Number x 1000	Kg
Sevin 4 L + Bud Nip	2.34 l + 14.0 l	0.00 a[2]	0.75 a	33.2 de	1391 b
Sevin 4 L + Royal MH	2.34 l + 14.0 l	0.25 a	0.00 a	18.2 ab	638 a
Orthene 75SP + Bud Nip	1.12 kg + 14.0 l	0.75 a	0.00 a	32.5 de	1247 b
Orthene 75SP + Royal MH	1.12 kg + 14.0 l	0.00 a	0.25 a	17.2 ab	522 a
Lannate 4L + Bud Nip	1.17 l + 14.0 l	0.25 a	0.00 a	31.4 cde	1373 b
Lannate 4L + Royal MH	1.17 l + 14.0 l	0.00 a	0.00 a	22.0 abc	504 a
Dipel WP + Bud Nip	1.12 kg + 14.0 l	1.25 bc	0.00 a	33.0 de	1388 b
Dipel WP + Royal MH	1.12 kg + 14.0 l	0.25 a	0.25 a	16.0 ab	471 a
Bud Nip	14.0 l	0.75 ab	7.50 b	25.7 bcd	1134 b
Royal MH	14.0 l	0.50 a	8.75 bc	13.4 a	476 a
Check-with Off-Shoot-T	-	0.00 a	11.25 bc	33.2 de	1372 b
Check-no Off-Shoot-T	-	2.25 c	12.00 c	41.5 e	1237 b

[1] All treatments except final check were sprayed with 4% Off-Shoot-T one week before treatment spraying.

[2] Means in the same column followed by the same letter are not significantly different (P=0.05) DMRT.

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Source: https://www.industrydocuments.ucsf.edu/docs/xtyk0000

C. Insect Pest Monitoring:

Cone-type wire traps baited with Virelure were monitored at Oxford, NC (6 traps) and Tifton, Georgia (10 traps) over the past 2 seasons. Data from these surveys were combined with past information on H. virescens population levels so that we could more accurately predict future peaks (Fig. 1). This information was used for better planning of field experiments and for determining optimum planting dates for host plant resistance field screens.

D. Effects of Soil-Incorporated and Transplant-Water Insecticides and Nematicides on Spined Stilt Bug, Jalysus wickhami Van Duzee, Survival:

(1) Fourteen formulations of 12 soil-incorporated or transplant-water-applied insecticides and nematicides were tested for their effects on stilt bugs in a field test at Oxford, NC. One hundred stilt bugs were introduced into each row box containing 3 treated tobacco plants (NC 2326) and left for one week. Three replications were set up for each treatment in a randomized complete block design. The boxes were then opened and the stilt bugs were counted. This experiment was repeated for plants 3 and 5 weeks after transplanting. The systemic materials such as Nemacur, Temik, Vydate, and Di-syston were particularly devastating to the stilt bugs (Table 3). Several materials had little impact on stilt bug survival.

(2) Nine treatments of transplant-water and soil-incorporated pesticides were tested for their effects on stilt bugs on potted tobacco (NC 2326) plants. Ten adult stilt bugs of mixed sex were enclosed inside glass lantern globes; five replications were conducted in each treatment. Live stilt bugs were counted on days 1, 2, 3, 6, 7 and 9 after they were introduced on the plants. Ridomil and Diazinon 14G had little effect on stilt bug survival (Table 4). Mocap, Dasanit, and Mocap killed all stilt bugs by 7 days after introduction, and Temik, Furadan, and Nemacur killed all stilt bugs shortly after they were put on treated plants (Table 4).

E. Dispersal of Spined Stilt Bugs From Central Release Zones in Flue-Cured Tobacco Fields in Bladen Co., NC:

The dispersal of spined stilt bugs from central release zones (RZ) in 6 flue-cured tobacco fields in Bladen Co. was investigated. Stilt bugs were reared at Oxford, NC in two large plastic greenhouses on potted tobacco plants and were fed previously frozen tobacco hornworm eggs. Adults were collected into plastic shoe boxes with 200 insects per container and stored overnight at 55°F. The following mornings stilt bugs were released into the RZ's which were 6 rows wide and 10 plants long. Bugs were released on every plant in the RZ. Plots radiating from the RZ were set up at regular intervals in 4 directions to 100 m from the RZ. Plots consisted of 10 plants in a row, and these were examined for stilt bugs daily. Three row-boxes covering 3 plants and containing 100 stilt bugs each were set up in each field. Four, 7, and 14 days after release they were examined for insect survival.

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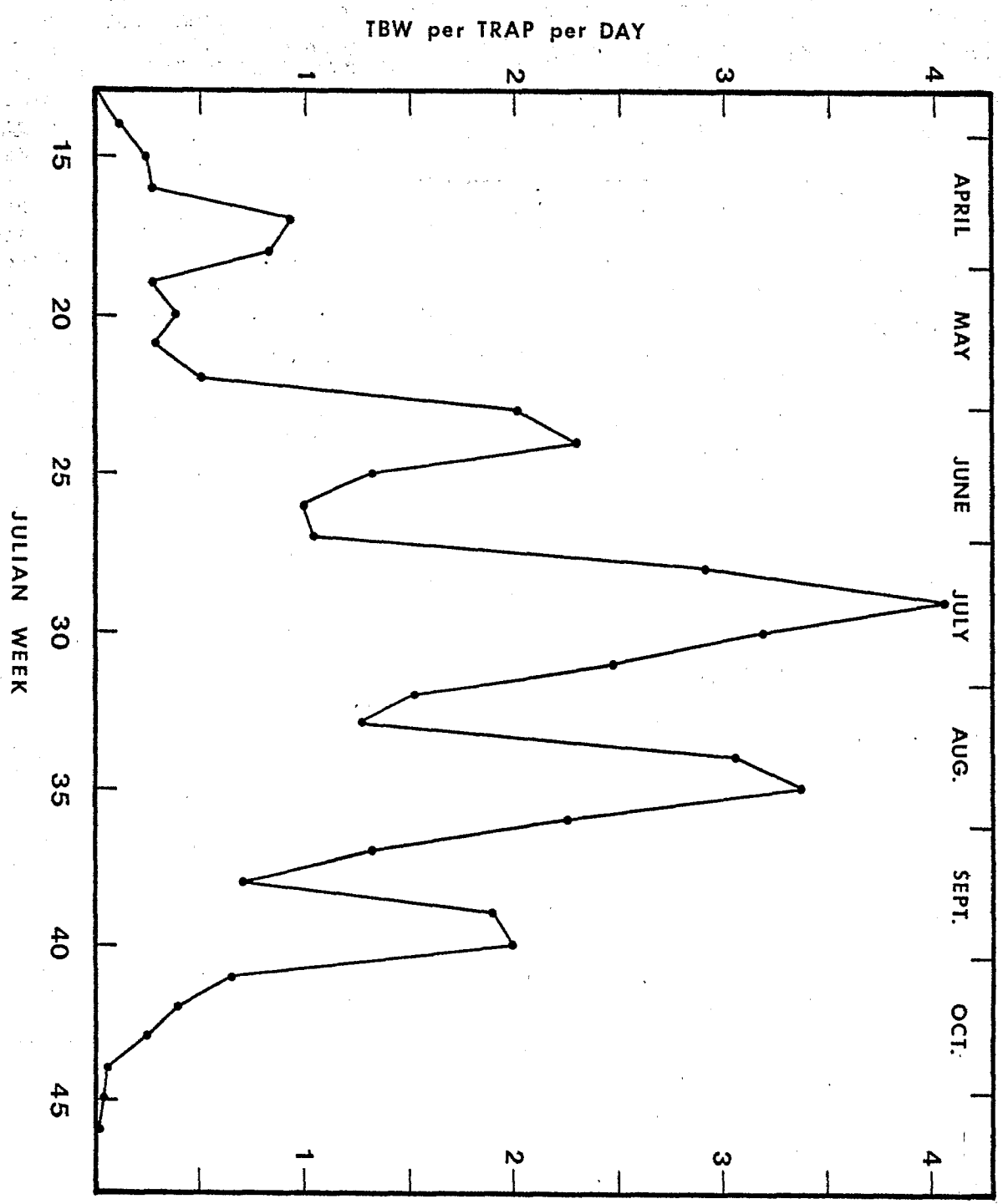

Fig. 1 - Average numbers of male tobacco budworm (TBW) moths collected per day in cone-type wire traps baited with Virelure at Gainesville, Florida (1978 and 1979) and Tifton, Georgia (1982 and 1983).

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Source: https://www.industrydocuments.ucsf.edu/docs/xtyk0000

Table 3. Survival of spined stilt bugs on flue-cured tobacco plants treated with various soil insecticides and nematicides at Oxford, NC, 1983.

Treatment	Rate per acre	Application[1] Method	Stilt Bugs Recovered Alive After One Week on Plants of Indicated Age After Transplanting[2] 3 weeks	5 weeks	Percent Recovery
Nemacur 3EC	2 gal.	SI	1	22	3.8
Temik 15G	20 lbs.	SI	5	28	5.5
Vydate L	1 1/4 pt.	TPW	27	65	15.3
Di-Syston 8	4 pt.	SI	43	67	18.3
Diazinon 50 WP	6 oz.	TPW	39	81	20.0
Furadan 10G	60 lbs.	SI	30	106	22.7
Dyfonate 4 EC	2 qt.	SI	58	88	24.3
Parathion 4 EC	3 qt.	SI	65	88	25.5
Diazinon 14 G	14 lbs.	SI	47	109	26.0
Orthene 75 SP	1 lb.	TPW	15	141	26.0
Mocap 6 EC	1 gal.	SI	32	124	26.0
Mocap 10 G	8 lbs.	SI	60	98	26.3
Dasanit SC	1 gal.	SI	32	146	29.7
Lorsban 4 EC	3 qt.	SI	72	112	30.7
Check (Field)	-	-	32	141	28.8
Check (Lab)	-	-	159	178	56.2

[1] TPW = Transplant Water
SI = Soil Incorporated 2 weeks before transplanting

[2] For each treatment, 100 stilt bugs were caged in each of 3 screened boxes that enclosed 3 flue-cured tobacco plants.

Table 4. Survival of spined stilt bugs in glass lantern globes enclosing potted tobacco plants treated with various soil insecticides and nematicides at Oxford, NC, 1983.

Treatment	Rate per acre	Application[1] Method	Live Stilt Bugs After Indicated[2] Days on Treated Tobacco Plants 1	2	3	6	7	9
Temik 15G	20 lb.	SI	1	0	0	0	0	0
Furadan 10G	60 lb.	SI	2	1	0	0	0	0
Nemacur 10G	60 lb.	SI	22	7	0	0	0	0
Mocap 6 EC	1 gal.	SI	30	14	8	6	0	0
Dasanit SC	1 gal.	SI	36	15	4	0	0	0
Orthene 75 SP	1 lb.	TPW	49	37	15	0	0	0
Diazinon 14 G	14 lb.	SI	50	48	48	28	25	11
Ridomil 2 E	2 qt.	TPW	49	49	49	46	42	25
Ridomil 2 E	2 qt.	SI	50	49	47	41	33	9
Check	-	-	50	50	50	43	38	10

[1] SI = Soil Incorporated one week before stilts were introduced.
TPW = Transplant Water

[2] Ten stilt bugs were put in each of 5 lantern globes.

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Source: https://www.industrydocuments.ucsf.edu/docs/xtyk0000

There was little movement away from the RZ's (Table 5). The average distance travelled per stilt bug peaked on day 9 after release at ca. 6 meters. Mortality was very high in all fields, and this was later attributed to the pre-plant soil-incorporated nematicide, Nemacur. Less than 10% of the expected number of stilt bugs were recovered after 2 days.

Table 5. Survival and distribution of spined stilt bugs released into 6 flue-cured tobacco fields in Bladen Co., NC, 1983

Days After Release[1]	Numbers of Stilt Bugs Found at Indicated Distance from Release Zone in Meters[2]							
	RZ	AP	5	10	20	30	40+	Total
0	9775	1531	45	1	0	0	0	11,352
1	6118	1730	168	8	0	0	0	8,024
2	2961	748	138	3	0	0	0	3,850
4	1657	500	195	1	0	0	0	2,353
6	1008	227	108	6	1	1	0	1,351
7	739	259	139	4	0	0	0	1,141
8	257	138	74	3	0	1	1	474
9	159	90	41	1	1	0	1	293
11	34	21	1	0	0	0	0	56
13	19	9	1	0	0	0	0	29
14	10	3	2	0	0	0	0	15
15	8	2	0	0	0	0	0	10
16	0	0	0	0	0	0	0	0

[1] 61,000 stilt bugs were released into 6 fields on 3 release dates (5-24, 6-1, and 6-22-83).

[2] RZ=Release Zone; AP=Plants Adjacent to Release Zone.

F. Effects of Cuticular Chemicals from Green Tobacco Leaves on Tobacco Budworm Oviposition:

The green leaves of commercially grown tobaccos are covered with sticky exudates that originate from glanded trichomes. This leaf surface material is composed of hydrocarbons, fatty alcohols, wax esters, isoprenoids, and other chemicals. Over 50% of the surface gum may be composed of macrocyclic diterpenoids known as duvanes. Four duvanes isolated from green tobacco are α- and β-4,8,13-duvatriene-1-ols and α- and β-4,8,13-duvatriene-1,3-diols. These compounds are known to possess biologial activity such as antifungal properties and plant growth inhibition. Our work shows that duvanes also affect tobacco budworm ovipositional behavior.

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Source: https://www.industrydocuments.ucsf.edu/docs/xtyk0000

There are some tobacco introductions (TI's) that have few glanded trichomes, and thus produce only small amounts of cuticular chemicals. One such tobacco, TI 1112, has consistently been resistant to tobacco budworm damage in small-plot field screenings over the past 12 years in North Carolina, South Carolina and Georgia. Other tobaccos low in leaf surface exudates also were resistant.

(1) Laboratory Cage Tests -

A cage (2.4 x 2.4 x 2.0 m) bioassay was developed to measure tobacco budworm ovipositional preferences. Treatments of four tobcco plants 5- to 7-weeks old were separated at opposite ends of 2.4 x 2.4 x 2.0 m screened cages located outside. Ten female moths (held for 2-3 days after eclosion with 10 males in mating cages) were released shortly before dark into the dimly lighted cages. The following morning all budworm eggs were counted, and data were utilized when at least 50 eggs were found and at least five of the moths in each cage were found successfully mated as determined by examination for spermatophores. Details of plant production, insect rearing, cage design, and other experimental techniques are described by Jackson et al. (1983).

Cuticular chemicals were removed from NC 2326 and sprayed onto TI 1112, a resistant tobacco. When given a choice between TI 1112 and a flue-cured control, NC 2326, moths lay ca. 75% of their eggs on NC 2326 (Jackson et al. 1983). Successful spray applications were made using a small paint air-brush (Badger Air-Brush Co., model 250). This apparatus had a ca. 20-ml reservoir, a teflon delivery tube, and was attached to a small compressor that would deliver up to 200 kilopascals (kPa). About 35 kPa was optimal for spraying the plants as it produced a fine mist that did not run off the leaves. Both upper and lower leaf surfaces were sprayed equally, as were all leaf positions. For sprayed controls, 0.5 ml of NC 2326 whole leaf washes (WLW) or methanol-water solubles (MWS) were mixed with 14.5 ml of a carrier solution (3:1 acetone:water) used to dilute the leaf washes so they could be sprayed. Control plants were sprayed with 0.5 ml solvent blank (SB) consisting of hexane:methylene chloride (3:1) in 14.5 ml of the same carrier used for the test plants. Diterpene treatments were also applied as 0.5 ml spray material (1 plant equivalent) in 14.5 ml of carrier.

The following diterpenes were bioassayed by the indicated experiments:

1. Mixture of α- and β-4,8,13-duvatriene-1,3-diols
   a. TI 1112 sprayed with α + β-diols vs TI 1112 with NC 2326 WLW (10 replications, 1982);
   b. TI 1112 sprayed with α + β-diols vs TI 1112 with SB (8 replications, 1982);
2. α-4,8,13-duvatriene-1-3-diol
   a. TI 1112 sprayed with α-diol vs TI 1112 with NC 2326 WLW (10 replications, 1982);
   b. TI 1112 sprayed with α-diol vs TI 1112 with NC 2326 MWS (14 replications, 1983);
   c. TI 1112 sprayed with α-diol vs TI 1112 with SB (20 replications, 1982-83);

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3. β-4,8,13-duvatriene-1,3-diol
   a. TI 1112 sprayed with β-diol vs TI 1112 with NC 2326 WLW (10 replications, 1982);
   b. TI 1112 sprayed with β-diol vs TI 1112 with NC 2326 MWS (15 replications, 1983);
   c. TI 1112 sprayed with β-diol vs TI 1112 with SB (20 replications, 1982-83);
4. Mixture of α- and β-4,8,13-duvatriene-1-ols
   a. TI 1112 sprayed with α + β-ols vs TI 1112 with NC 2326 WLW (10 replications, 1982);
   b. TI 1112 sprayed with α + β-ols vs TI 1112 with NC 2326 MWS (15 replications, 1983);
   c. TI 1112 sprayed with α + β-ols vs TI 1112 with SB (20 replications, 1982-83);
5. TI 1112 sprayed with Cis-abienol vs TI 1112 with SB (12 replications, 1983);
6. TI 1112 sprayed with wax esters vs TI 1112 with SB (12 replications, 1983);
7. TI 1112 sprayed with $C_{22}OH$ vs TI 1112 with SB (12 replications, 1983);
8. TI 1112 sprayed with sucrose esters vs TI 1112 with SB (12 replications, 1983).

Concurrently, unsprayed TI 1112 and NC 2326 were tested in no-choice and free-choice tests, which served as controls (Jackson et al., 1983).

Prior to individual analyses of variance for each experiment, data were transformed first to a percentage of the total eggs counted per replication (X) and then transformed to arcsine (square root X). Preference for egg laying on a particular treatment versus the control was evaluated by a t-test.

To determine the efficiency of the spray-back procedure, cuticular leaf chemical samples were taken from sprayed and unsprayed tobaccos. After counting the budworm eggs 16-24 hr post-spraying, one 10 to 20-cm long leaf from each of the four plants within a treatment was collected and dipped in CH_2Cl_2. The cuticular chemical samples were analyzed by GC as described by Severson et al. (1984).

Initially, it was determined that NC 2326 WLW would stimulate oviposition onto TI 1112. Later, we found that the active compounds were in the methanol-water soluble fraction of the NC 2326 WLW, and the hexane soluble fraction was inactive. In 1983 we tested all 4 duvanes individually and found they each stimulated tobacco budworm oviposition onto TI 1112 (Table 6). Four other classes of compounds (docosanol, wax esters, sucrose esters, and Cis-abienol) did not affect egg laying (Table 6).

(2) Field Experiments -

The activities of tobacco cuticular diterpenes were tested in the field at Oxford, NC and Florence, SC during 1983. Five replications of six treatments of 6-week-old tobacco plants were arranged in a cross pattern in fields isolated from other tobacco. The six treatments were:

1. TI 1112 with SB
2. TI 1112 with NC 2326 MWS
3. TI 1112 with α-diol

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Source: https://www.industrydocuments.ucsf.edu/docs/xtyk0000

Table 6. Tobacco budworm oviposition in screen cages on potted tobacco plants sprayed with chemicals extracted from tobacco leaf surfaces.

Experiment number	Years tested	Replications	Avg.no. eggs/rep	Tobacco Entries	Treatment[1]	Percent of total eggs	T-test[2]
1	1980-83	109[3]	236.0	A-NC2326 B-TI1112	None None	75.2 24.8	**
2	1981	10	184.4	A-TI1112 B-TI1112	None None	53.3 46.7	N.S.
3	1980-82	27	214.7	A-TI1112 B-TI1112	NC2326 WLW SB	76.5 23.5	**
4	1982-83	20	203.8	A-TI1112 B-TI1112	NC2326 MWS SB	74.8 25.2	**
5	1982	10	203.8	A-TI1112 B-TI1112	NC2326 WLW α-Diol	44.5 55.5	N.S.
6	1983	14	283.7	A-TI1112 B-TI1112	NC2326 MWS α-Diol	49.6 50.4	N.S.
7	1982-83	20	257.9	A-TI1112 B-TI1112	α-Diol SB	64.2 35.8	**
8	1982	10	229.0	A-TI1112 B-TI1112	NC2326 WLW β-Diol	40.2 59.8	N.S.
9	1983	15	207.5	A-TI1112 B-TI1112	NC2326 MWS β-Diol	54.0 46.0	N.S.
10	1982-83	20	441.4	A-TI1112 B-TI1112	β-Diol SB	63.3 36.7	**
11	1982	10	314.3	A-TI1112 B-TI1112	NC2326 WLW α + β-Diols	35.1 64.9	*
12	1982	8	364.4	A-TI1112 B-TI1112	α + β-Diols SB	72.2 27.8	*
13	1982	10	191.0	A-TI1112 B-TI1112	NC2326 WLW α + β-ols	49.3 50.7	N.S.
14	1983	15	186.1	A-TI1112 B-TI1112	NC2326 MWS α + β-ols	51.3 48.7	N.S.
15	1982-83	20	185.7	A-TI1112 B-TI1112	α + β-ols SB	65.6 34.4	**
16	1983	12	191.8	A-TI1112 B-TI1112	$C_{22}OH$ SB	50.5 49.5	N.S.
17	1983	12	164.6	A-TI1112 B-TI1112	Cis-Abienol SB	42.2 57.8	N.S.
18	1983	12	211.4	A-TI1112 B-TI1112	Wax Esters SB	45.2 54.8	N.S.
19	1983	12	185.3	A-TI1112 B-TI1112	Sucrose Esters SB	62.1 37.9	N.S.

[1]0.5 ml of NC 2326 whole leaf wash (WLW), NC 2326 methanol-water soluble fraction (MWS), α-Diol (α-4,8,13-duvatriene-1,3-diol), β-Diol (β-4,8,13-duvatriene-1,3-diol), α + β-Diols, α + β-ols (mixture of α- and β-4,8,13-duvatriene-1-ols), Cis-Abienol, wax esters, sucrose esters, $C_{22}OH$, or Solvent Blank (SB) applied per plant in 14.5 ml carrier (3:1 acetone:H_2O).

[2]N.S.=Not Significant; *=Significant at 5% level; **=Significant at 1% level.

[3]Data from Jackson et al. (1984) included.

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4. TI 1112 with β-diol
5. TI 1112 with α + β-ols
6. NC 2326 with SB

The centers of adjacent replications were 10 m apart. Within a replication, the treatments were positioned at the points of an equilateral hexagon with the nearest plants of unequal treatments being ca. 2.5 m apart. Four plants per treatment per replication were arranged in a square with 0.5 m between plants. The Oxford and Florence tests had similar designs, except that 6-week-old potted plants (2 weeks in greenhouse and 2 weeks in shade outdoors) were used at Oxford and 6-week-old field-grown plants were used at Florence. One spraying per test was made. The Oxford plants were sprayed in the same way as plants for cage tests, starting at 1 PM on August 23. After the plants had dried, they were moved to a recently mowed stubble field of harvested wheat and arranged in the pattern described above. This field was ca. 500 m from the nearest tobacco field. Two hundred mated female budworm moths (40/ replication) were released per night for four successive nights. Eggs were counted and removed on four successive days. The design at Florence was similar, and this test was sprayed on July 7.

Since the experimental designs were the same, data from the Oxford and Florence tests were transformed to log (X + 1.0) and combined prior to analysis of vraiance. Treatment means were then separated by Duncan's New Multiple Range Test.

The field spray-back tests were sampled for chemical analyses using a leaf plug technique. One 2-cm diam leaf plug was taken from each plant as soon as they had dried (Day 0) and at 1, 2, 3, and 4 days after spraying. The cuticular chemicals were removed by dipping each of the 4 plugs per sample eight times into a 20-ml scintillation vial containing 10 ml CH_2Cl_2. The vial was capped with a teflon-lined cap and immediately frozen. Samples were transferred to Athens, GA, under dry ice, and stored at-17.8°C. After warming to room temperature, heptadecanol internal standard was added, mixed, ca. 5 ml was removed, and solvent was removed using a nitrogen blowdown system in preparation for GC analysis (Severson et al. 1984).

As with the cage bioassays all the duvane diterpenes stimulated oviposition onto sprayed TI 1112 plants (Table 7), but activity declined rapidly after the first day. Levels of duvanes were nearly zero by 3 days after spraying.

G. Correlations Between Tobacco Leaf Surface Chemistry and Tobacco Insect Field Levels, Ovipositional Preference, Development, and Survival:

This was the third year of the 3-year experiment called the "Screening Correlation Test". Sixty-seven tobacco entries were screened over the last 3 years for insect resistance by 3 methods: (a) field plots of 12-plants at Tifton, GA and Oxford, NC; (b) oviposition cage (2.4 x 2.4 x 2.0 m) tests to determine tobacco budworm ovipositional preference; and (c) feeding tests of tobacco budworm larvae caged on intact tobacco seedlings. Leaf surface chemical samples were washed from leaf plugs of 6-week-old tobaccos with methylene chloride in all tests. Profiles of cuticular chemicals from each entry were determined by co-workers at the Russell Research Laboratory, USDA,

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Table 7. Tobacco budworm oviposition in 1983 field tests on 6-week-old TI 1112 or NC 2326 plants sprayed with duvane diterpenes, the methanol-water soluble fraction of the NC 2326 whole leaf cuticular wash, or a solvent blank.

Treatment[1]	Average number of eggs/plant on indicated day after spraying[2]			
	1	2	3	4
TI 1112 with SB	4.9 a	7.0 a	7.7 a	10.8 a
TI 1112 with β-Diol[3]	7.7 b	12.4 b	11.8 b	10.3 a
TI 1112 with α + β-ols[3]	9.7 bc	10.4 b	19.4 b	8.4 a
TI 1112 with NC 2326 MWS	15.9 d	12.7 b	12.3 b	10.7 a
TI 1112 with α-Diol[3]	12.4 c	16.1 b	21.3 b	9.9 a
NC 2326 with SB	20.0 d	25.4 c	25.4 c	9.8 a

[1] 0.5 ml of NC 2326 methanol-water solubles (MWS), solvent blank (SB), or duvane diterpenes applied per plant in 14.5 ml carrier (3:1 acetone:H_2O).

[2] Data combined from Oxford, NC and Florence, SC tests; Means in the same column followed by the same letter are not significantly different at the 5% level, DMRT.

[3] α-diol = α-4,8,13-duvatriene-1,3-diol
β-diol = β-4,8,13-duvatriene-1,3-diol
α + β-ols = mixture of α- and β-4,8,13-duvatriene-1-ols

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ARS, Athens, GA. Quantitative levels of individual chemicals were then correlated to indices of resistance determined from various screening tests. For the 67 entries, there was a strong positive correlation (R^2=0.74) between the number of tobacco budworm eggs laid on the test entries and the logarithm of the level of total duvane diterpenes found on their leaf surfaces (Fig. 2). The duvanes are composed primarily of α- and β-4,8,13-duvatriene-1-ols and α- and β-4,8,13-duvatriene-1,3-diols. There also was a negative correlation between tobacco flea beetles and green peach aphids for several entries, although this relationship was not significant over all 67 entries. Feeding test data are incomplete and will be completed in 1984.

H. Effects of Lighting on Growth Parameters and Leaf Surface Chemistry of Greenhouse-Grown, Potted Tobacco Plants:

The quality of greenhouse-grown tobacco plants is generally too poor to do host plant resistance screening experiments. These plants are typically high in water content, low in leaf surface chemicals, and thinner than field-grown tobacco. We grew seven tobacco entries with divergent leaf surface chemical profiles under 6 lighting treatments in greenhouses. The tobacco entries were: NC 2326, TI 1112, TI 165, TI 1068, TI 1223, TI 1341, and TI 1406. The lighting treatments were: (1) Supplemental metal halide lighting in a glass greenhouse (=MH), (2) Supplemental high pressure sodium lighting in a glass greenhouse (=HPS), (3) Supplemental Flourescent-Incandescent lighting in a glass greenhouse (=FI), (4) Supplemental ultraviolet lighting in a glass greenhouse (=UV), (5) Glass greenhouse with no additional lighting (=GGH), and (6) Plastic greenhouse with no additional lighting (=PGH). Five plants of each tobacco entry was grown under each lighting regime (14:10 hr Light:Dark) and the experiment was repeated 3 times. Plants were sampled 5-6 weeks post-transplanting on March 3, April 12, and May 19, 1983. All leaves were removed, weighed, and areas determined. One leaf per plant was sampled for leaf surface chemistry by washing ca. 2-cm diameter leaf plugs in 10 ml methylene chloride in scintillation vials. All leaves and stems were dried at 95°C for 24 hr to determine dry weights. Plants grown with high light intensities under either metal halide or high pressure sodium lamps were similar to field-grown plants in physical characteristics and leaf surface chemistries (Table 8).

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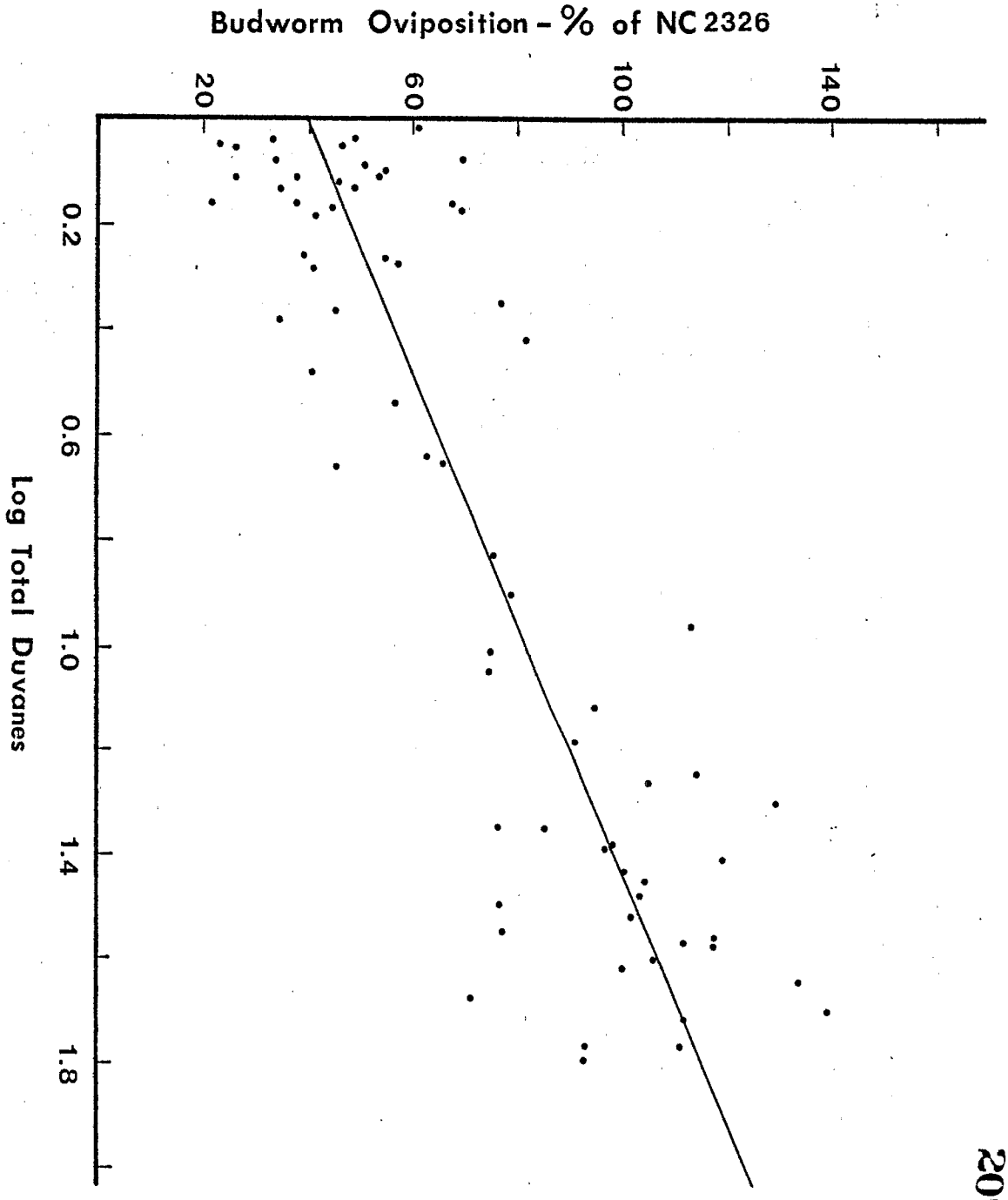

Fig. 2 - Correlation between the logarithm of levels of total duvane diterpenes found on the leaf surface of 67 tobacco entries and a relative measure of tobacco budworm oviposition using NC 2326 as the standard. Regression equation was $Y = 39.8 \pm 41.4X$; $R^2 = 0.74$; $N = 67$.

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Table 8. Growth parameters and leaf surface chemistry of 7 tobacco entries grown under 6 light regimes in greenhouses at Oxford, NC, 1983.

Light[1] Regime	Tobacco Entry	Total Leaf Area per Plant in cm^2	Total Green Weight per Plant in in gms.	Total Dry Weight per Plant in gms.	Weight Dry Leaf per 1000 cm^2 leaf area	Cuticular Chemicals - $\mu g/cm^2$				
						Duvane Diterpenes[2]		Labdane[3] Diterpenes	Docosanol	Sucrose esters
						α + β mono-ols	α + β Diols			
MH	All[4]	582	22.7	3.1	4.8	10.3	30.0	5.3	0.31	12.7
HPS	All	643	26.3	3.7	5.2	10.5	34.0	6.3	0.36	15.6
UV	All	1032	35.5	3.7	3.1	5.8	16.6	3.0	0.16	7.3
FI	All	936	31.0	2.9	2.7	4.7	16.7	2.8	0.16	6.6
GGH	All	769	26.7	1.9	2.2	3.6	10.0	1.8	0.08	4.0
PGH	All	718	24.9	2.6	3.1	6.6	19.5	3.4	0.13	7.0
All[5]	NC 2326	744	28.2	3.2	3.9	0.5	32.6	0.0	0.26	0.0
All	TI 1112	748	25.2	2.6	3.2	0.1	0.6	0.0	0.01	0.1
All	TI 165	962	31.6	3.4	3.4	0.6	46.1	0.0	0.21	16.1
All	TI 1068	641	24.9	2.7	3.8	1.3	41.0	12.5	0.34	27.4
All	TI 1223	708	26.2	3.1	3.5	11.2	1.6	13.8	0.24	4.3
All	TI 1341	875	31.0	3.4	3.5	34.3	25.3	0.0	0.12	12.5
All	TI 1406	781	28.0	2.7	3.3	0.5	0.8	0.1	0.21	1.5

[1] MH = Metal Halide; HPS = High Pressure Sodium; UV = Ultraviolet; FI = Flourescent-Incandescent; GGH = Glass greenhouse; PGH = Plastic greenhouse.

[2] α- and β-4,8,13-duvatriene-1-ols and α- and β-duvatriene-1,3-diols.

[3] (12,Z)-/Labda-12-14-diene-8-ol(cis-Abienol) and (13,E)-/Labda-13-ene-8-15-diol (labdene-diol).

[4] Data summed over all tobacco entries.

[5] Data summed over all light regimes.

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II. Graduate Students: None

III. Postdoctoral Fellows: None

IV. Publications:

A. JACKSON, D. M. 1983. Insect control on flue-cured tobacco with Bacillus thuringiensis var. kurstaki spray formulations, 1982. Insect. Acar. Tests 8:223

B. JACKSON, D. M. 1983. Control of tobacco budworms and tomato hornworms with Bacillus thuringiensis var. kurstaki, 1981. Insect. Acar. Tests 8:224.

C. JACKSON, D. M., J. S. CHEATHAM, J. M. PITTS, and A. H. BAUMHOVER. 1983. Ovipositional response of tobacco budworm moths (Lepidoptera:Noctuidae) to tobacco introduction 1112 and NC 2326 in cage tests. J. Econ. Entomol. 76:1303-8.

D. SOUTHERN, P. S. and D. M. JACKSON. 1984. Control of Heliothis virescens on flue-cured tobacco using Bacillus thuringiensis and a cottonseed flour feeding stimulant. Tob. Sci. 28:10-13

V. Manuscripts Accepted for Publication:

A. JACKSON, D. M., F. C. TINGLE, and E. R. MITCHELL. 1984. Survey of Heliothis spp. larvae found on Florida beggarweed and post-harvest tobacco in Florida. Fla. Entomol. 67:(In Press).

B. SEVERSON, R. F., R. F. ARRENDALE, O. T. CHORTYK, A. W. JOHNSON, D. M. JACKSON, G. R. GWYNN, J. F. CHAPLIN, AND M. G. STEPHENSON. 1984. Quantitation of cuticular components of green tobacco leaf. J. Agric. Food Chem. (In Press).

C. JACKSON, D. M. 1984. Insect and sucker control with tank mix combinations of four insecticides and two sucker control chemicals on flue-cured tobacco, 1983. Insect. Acar. Tests 9: (In Press)

D JACKSON, D. M. 1984. Control of insects on flue-cured tobacco with Bacillus thuringiensis var. kurstaki spray formulations, 1983. Insect. Acar. Tests 9: (In Press)

VI. Manuscripts in Review:

A. JACKSON, D. M., R. F. SEVERSON, A. W. JOHNSON, J. F. CHAPLIN, and M. G. STEPHENSON. Ovipositional response of tobacco budworm moths (Lepidoptera:Noctuidae) to cuticular chemical isolates from green tobacco leaves. (Submitted to Environ. Entomol.).

B. JACKSON, D. M. and E. R. MITCHELL. Growth and survival of tobacco budworm (Lepidoptera:Noctuidae) larvae on Florida beggarweed (Fabaceae). (Submitted to J. Econ. Entomol.).

VII. Papers Presented at Professional Meetings:

A. JACKSON, D. M., R. F. SEVERSON, J. S. CHEATHAM, A. W. JOHNSON, and G. A. HERZOG. 1983. Stimulatory effects of leaf-surface diterpenes from green tobacco on Heliothis virescens oviposition. Paper No. 490, Entomol. Soc. Am. Annu. Meeting, Nov. 28 - Dec. 2, 1983. Detroit, MI.

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B. JACKSON, D. M., R. F. SEVERSON, A. W. JOHNSON, G. HERZOG, M. G. STEPHENSON, J. F. CHAPLIN, and G. R. GWYNN. 1984. Relationship between levels of cuticular diterpenes from several tobacco entries and observed tobacco budworm resistance. Sixth Biennial Plant Resistance to Insects Workshop. Feb. 21-23, 1984, Charleston, SC.

C. CHAPLIN, J. F., D. M. JACKSON, R. F. SEVERSON, M. G. STEPHENSON and G. A. HERZOG. 1984. Effect of reciprocal grafts on insect resistance and chemical composition in tobacco. Sixth Biennial Plant Resistance to Insects Workshop. Feb. 21-23, 1984, Charleston, SC.

D. GWYNN, G. R., R. F. SEVERSON, and D. M. JACKSON. 1983. Inheritance of leaf surface chemicals in tobacco and relationship to insect resistance. 37th Tobacco Chemists' Res. Conf., Oct. 10-13, 1983, Wash., DC.

E. SEVERSON, R. F., R. F. ARRENDALE, O. T. CHORTYK, M. G. STEPHENSON, A. W. JOHNSON, and D. M. JACKSON. 1983. The isolation and characterization of the cuticular fatty alcohols and wax esters of green tobacco. 35th Annual SE Regional Meeting, Amer. Chem. Soc., Div. Agric. Food Chem., Nov. 8-11, 1983. Charlotte, NC.

F. SEVERSON, R. F., D. M. JACKSON, G. R. GWYNN, A. W. JOHNSON, M. G. STEPHENSON, and G. A. HERZOG. 1984. Analysis of tobacco cuticular chemicals related to host plant resistance to insects. Sixth Biennial Plant Resistance to Insects Workshop. Feb. 21-23, 1984, Charleston, SC.

G. STEPHENSON, M. G., J. F. CHAPLIN, D. M. JACKSON, R. F. SEVERSON, A. W. JOHNSON, G. A. HERZOG, and G. R. GWYNN. 1984. Techniques for field screening tobacco entries for host plant resistance to insects. Sixth Biennial Plant Resistance to Insects Workshop. Feb. 21-23, 1984, Charleston, SC.

VIII. Graduate Student Thesis: None

IX. Acknowledgements:

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THE USE OF FEEDING DETERRENTS IN PEST MANAGEMENT OF SOIL INSECTS IN TOBACCO

F. Gould, Project Leader

I. Summary of Research:

This project has the following objectives:

A. To identify natural feeding deterrent of wireworms through the laboratory screening of crude extracts obtained from plant species common in North Carolina.

B. To screen effective deterrents for deleterious side effects on tobacco (loss of vigor, leaf weight loss and lower growth rate) in the greenhouse.

C. To explore various methods of application of effective deterrent extracts to facilitate their incorporation into existing transplanting procedures.

D. To narrow the possible chemical classification of the active component(s) of effective crude extracts to increase the efficiency of future plant screenings.

The screening of 105 crude plant extracts revealed 6 plants which significantly reduced wireworm feeding damage to food baits in the laboratory. Intensive testing of the most promising extract, Asclepias tuberosa (butterfly milkweed) gave the most dramatic results with wireworms choosing untreated baits over treated baits 31 to 1. Crude milkweed extract applied to the roots and stems of tobacco at the transplant stage caused no mortality or loss of plant vigor (as measured by leaf weight, plant height or growth rate) when compared to untreated tobacco transplants. One possible application technique of feeding deterrents is their addition to the transplant water; for this technique to be effective, A. tuberosa must remain effective when diluted. Our studies have shown that diluting this crude extract with equal amounts of distilled water does not significantly reduce its anti-feedant properties. Eight-fold dilutions of S. tuberosa extract continued to significantly reduce wireworm feeding damage when compared to wireworm damage to untreated food baits. It is possible that a two-pronged attack (direct application of deterrent to transplants and addition of small amounts of deterrent to transplant water) may be the most effective means of wireworm control in the field.

Chemical analysis of crude A. tuberosa extract showed a high resinous component and the presence of di and tri terpenoids. Terpenoids have been implicated in the literature as having insect deterring properties. The screening of other members of the genus Asclepias revealed that A. incarnata (swamp milkweed) has wireworm deterring properties similar to A. tuberosa. The literature suggests

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that both A. tuberosa and A. incarnata use similar chemical classes as defense mechanisms and may point the direction for identification of plant species from other plant groups with feeding deterrent potential.

II. Graduate Students:

Villani, Michael (Ph.D. completion expected April 1984). Dissertation: Screening of crude plant extracts as feeding deterrents of soil insects.

Landis, Douglas (began studies for Ph.D., January 1984). Not yet decided.

III. Postdoctoral Fellows:

None

IV. Publications:

None

V. Manuscripts Accepted for Publication:

Villani, M. G. and F. Gould. 1984. Butterfly milkweed extract as a feeding deterrent of the wireworm, Melanotus communis Gyll. Entomol. Exp. Appl.

VI. Manuscripts in Review:

Villani, M. G. and F. Gould. Screening of crude plant extracts as feeding deterrents of the wireworm, Melanotus communis. (Submitted Jan. 1, 1984 to Entomol. Exp. Appl.)

Villani, M. G. and F. Gould. Defensive compounds in the genus Asclepias: an alternative to toxic cardenolides. (Submitted for internal review Feb. 1, 1984).

Villani, M. G. and F. Gould. The use of radiographs for movement analysis and life-history studies of soil insects. (Submitted for internal review, Feb. 15, 1984)

VII. Papers Presented at Professional Meetings:

Villani, M. G. December 1983. The use of plant extracts for soil insect control. National Meeting, Entomological Society of America, Detroit, Michigan.

Villani, M. G. and F. Gould. 1983. The use of crude plant extracts for wireworm control. Southeastern Branch Meeting, Entomological Society of America, Little Rock, Arkansas.

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VIII. Graduate Student Theses Completed During Reporting Period:

None

IX. Acknowledgements:

We would like to thank the many extension agents who allerted us to wireworm infestations and the growers who allowed us to collect wireworms from their fields during 1983.

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NC03699: PESTICIDE RESIDUES IN TOBACCO, TOBACCO PRODUCTS, AND MAIN-STREAM SMOKE

T. J. Sheets, Project Leader

I. Summary of Research:

Methiocarb Residues. Analyses of samples from a residue study with methiocarb conducted during the 1981 season were completed in 1983. Total residues consisting of methiocarb and two metabolites (the sulfoxide and the sulfone) were 0.62 and 1.05 ppm in first-harvest samples of flue-cured tobacco treated four times during the growing season with 1.0 and 2.0 kg/ha a.i. (season totals of 4.0 and 8.0 kg/ha) of methiocarb. Residues were progressively lower in samples from higher stalk positions. The residue was in the form of the sulfoxide and sulfone; the parent material, methiocarb, was below the lowest detectable limit in all samples. The sulfoxide was found in greater amounts than the sulfone in most samples. Methiocarb treatment had no effect on reducing sugar and total alkaloid contents.

Synthetic Pyrethroids. Sample analysis for the 2-year study with the synthetic pyrethroids, fluvalinate and permethrin, was completed in 1983. The results for fluvalinate were given in the report for 1982. When 0.2 kg/ha of permethrin were applied to mature leaves, residues immediately after application averaged 9.6 ppm on green flue-cured tobacco and declined to 7.0 ppm after 12 days. Residues averaged 10 ppm on cured leaves that were harvested 7 days after application and flue-cured. When losses in weight (mostly water) during curing were considered along with the residue present before and after curing, the results suggested a drop in the permethrin residue of 60 to 65% during the curing process.

EBDC and ETU. Delays were encountered in adapting a gas-chromatographic method for the analysis of EBDC and the metabolite ETU in tobacco, and some effort was devoted to a new approach using the High Performance Liquid Chromatograph (HPLC). The HPLC method was not sufficiently sensitive for residue determinations. Another gas-chromatographic method has been evaluated and can be used to analyze tobacco samples for EBDC and ETU. However, EBDC and ETU must be determined in separate analyses. A fluorinated derivative, to which the electron-capture detector is highly responsive, is formed with both EBDC and ETU. The lowest detectable concentration of the combined residue in tobacco is about 0.05 ppm. Samples were collected by a cooperator (Dr. P. B. Shoemaker) during 1983 from burley plots at Waynesville and Laurel Springs, NC. Analysis of samples is in progress.

Metalaxyl and MH. Studies were continued in 1983 to determine if metalaxyl and MH interact to alter residue levels from those resulting when the two pesticides are used separately on flue-cured tobacco. Samples from the 1982 plots were analyzed in 1983; analyses of 1983 samples have not been completed. The 1982 results indicate that metalaxyl did not alter the MH residue. At one of two locations, residues of metalaxyl, applied four times as a foliar spray followed by MH, appeared higher than those for the same metalaxyl treatment without MH.

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MH in 1983 Tobacco Products. Tobacco products were sampled on the retail market in Raleigh, N.C. over an 8-month period in 1983. Analyses of samples for MH have been completed. MH residues ranged from 44 to 68 ppm with an average of 59 ppm for 19 brands of U.S. cigarettes. Previous averages were 47, 47, 48, and 50 ppm for 1975, 1977, 1979, and 1981, respectively. Residues in cigars, pipe tobacco, chewing tobacco and snuff were about the same as in previous years and averaged 11, 26, <10, and 28 ppm, respectively. Analysis of products for other pesticides is in progress.

Effect of Overhead Irrigation on MH. Light (0.26 cm) and moderate (2.2 cm) overhead irrigations were applied to flue-cured tobacco at 4, 8, 12, and 24 hr after application of 2.5 kg/ha (1 x rate) of MH. Subsequently, selected plots were retreated with 0.62 (0.25 x) and 1.25 (0.5 x) kg/ha. Sucker control was recorded and those results are reported by Dr. H. Seltmann. Residue analyses are in progress.

Free and Bound MH. Plots were established on the Central Crops Research Station near Clayton, N.C. in the 1983 season. The K-MH treatments were applied at 4-hr intervals over a 24-hr period. Samples of green leaves were taken 24 hr after MH application for residue analysis. Cured leaf samples after normal harvest and curing were also saved for analysis. Free and bound MH residues are now being determined on green and cured samples.

II. Graduate Students:

Meyer, Susan A., M.S., Toxicology
Thesis title: Free and Bound MH in Flue-Cured Tobacco as Affected by Time of Spraying
Requirements for the degree to be completed by August 1983.

III. Publications:

Elkins, Phyllis D., T. J. Sheets, and R. B. Leidy. 1983. Transfer of disulfoton and three metabolites to mainstream smoke. Tob. Sci. 27:142-145.

IV. Manuscripts in Review:

Sheets, T. J. and H. Seltmann. Residue and sucker control from two formulations of MH. Submitted to Beitrage zur Tabakforschung.

Sheets, T. J., R. B. Leidy, and W. J. Mistric. Residues of methiocarb in flue-cured and burley tobacco. Prepared for Tobacco Science.

Leidy, R. B. and T. J. Sheets. Pyrethroid residues in flue-cured tobacco. Prepared for Beitrage zur Tabakforschung.

Hunt, T. W., G. W. Wojeck, and T. J. Sheets. Exposure of applicators to maleic hydrazide (MH). Prepared for Bull. Environ. Contam. Toxicol.

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Herman, N. D., T. W. Hunt, and T. J. Sheets. Exposure of hand harvesters to maleic hydrazide (MH) in the field. Prepared for Bull. Environ. Contam. Toxicol.

V. Papers Presented at Professional Meetings:

Pyrethroid residues in flue-cured tobacco by HPLC - A two-year study. R. B. Leidy, T. J. Sheets, and J. B. Weber, Southeastern Section, Amer. Chem. Soc., Charlotte, N.C. Dec. 10, 1983.

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TOBACCO GENETICS

3504 GENETIC INVESTIGATIONS IN NICOTIANA SPECIES POPULATIONS

E. A. Wernsman and D. F. Matzinger

I. Summary of Research:

A. Evaluation of Dwarf Mammoths

Long-term recurrent selection studies to determine the inheritance of plant height and number of leaves have shown that the two traits are genetically correlated with each other and with yield. Selection of plants with reduced plant height has led to plants with reduced numbers of leaves. A stable dwarf line was isolated which flowered within two weeks following transplanting at a height of 20 cm and which produced four leaves. Studies have been initiated to evaluate the potential use of combining dwarf lines with mammoth genotypes to modify agronomic properties of the plant.

The dwarf line was crossed with mammoth SC58 and selections were made for non-flowering, short plants. Four F_4 lines were isolated which produced these characteristics. They were non-flowering until late in the summer and when topped to a normal leaf number they were shorter than standard varieties. The four lines were evaluated at Clayton in 1982 and at Whiteville in 1983. Each test included NC95 and NC13 as an average yielding and high yielding check. Two plots of each mammoth line were included within a replication. One was topped to the same leaf number as the checks and the other was topped at four additional leaves. The average leaf number of the checks at Clayton was 18 and at Whiteville was 20. At the same time that the checks were topped, the plots of the mammoth dwarfs were topped to 18 leaves (N treatment) or 22 leaves (H treatment) at Clayton and 20 (N) or 24 (H) leaves at Whiteville.

Performance data averaged over locations for the two check varieties and the mammoths (numbered 1 to 4) at normal and high leaf number topping are presented in Table 1. No significant differences were observed among the four lines within a topping treatment for any characters except plant height, where Mam 1 is slightly shorter and Mam 4 is slightly taller than the others at both topping treatments. Plant height of the mammoths topped to the same number of leaves as the checks was 96 cm, about 23% shorter than the checks. When the mammoths were topped to four additional leaves, they averaged 109 cm, 12% below the checks. The normal topped mammoths yielded about the same as NC95 and the high topped mammoths yielded similar to NC13. The mammoths within both topping treatments did not differ among themselves for grade index, reducing sugars, leaf length, or leaf width, and were in the range of the check varieties.

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Table 1. Performance of dwarf-mammoth lines at two topping levels.

Entries	Leaves No.	Height cm	Yield lb/A	Grade Index	Alkaloids %	Sugars %	L.Length cm	L.Width cm
NC95	19	125	3133	66.9	2.75	19.6	59.1	31.7
NC13	19	124	3684	78.9	2.62	20.3	65.6	37.2
Mam 1N	19	92	3213	74.4	1.98	16.6	61.0	31.0
Mam 1H	23	106	3603	66.5	1.83	16.3	60.7	31.2
Mam 2N	19	97	3342	76.4	1.72	15.6	61.2	31.9
Mam 2H	23	108	3685	72.7	1.66	16.8	61.2	31.7
Mam 3N	19	96	3267	73.3	1.95	17.9	60.0	30.6
Mam 3H	23	111	3790	76.4	1.67	17.5	60.1	30.2
Mam 4N	19	100	3333	76.6	1.87	16.9	60.2	31.0
Mam 4H	23	112	3559	73.2	1.72	17.3	59.9	30.1
$N\bar{x}$	19	96	3288	75.2	1.88	16.8	60.6	31.1
$H\bar{x}$	23	109	3659	72.2	1.72	17.0	60.5	30.8
N vs H		**	**	N.S.	**	N.S.	N.S.	N.S.
Within		*	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

It appears that dwarf genes can be combined with the mammoth character to reduce the plant height of tobacco and by modifying the number of leaves left on the plant can produce tobacco similar to conventional tobacco. The exception in this experiment is total alkaloids. The alkaloids of all of the mammoth selections were about 33% lower than the checks. It is possible that the dwarf parent used in this study has a low alkaloid potential. Alkaloid levels of dwarf lines cannot be measured per se since the lines have only about four leaves and all accumulate alkaloids, but their genetic capacity to produce alkaloids when in a high yielding background may be masked. One will probably need to test cross potential dwarf parents to a normal tobacco variety to establish those with the ability to transmit alkaloid producing genes.

B. Total Particulate Matter, Total Alkaloid Index

The eighth cycle of selection to reduce the ratio of particulate matter index (PMI) to total alkaloids (TA) was conducted in 1983. Second priming leaves of 400 plants produced from selections in the previous generation were evaluated for PMI, TA, and reducing sugars. Means of the 400 plants are presented in Table 2. A restricted selection index was applied to each plant and 60 were selected as parents for the next generation. The selected plants were 17.4% below the population mean for PMI and 14.7% above the mean for total alkaloids. Parent plants selected on the basis of the index were about 4% below the population mean for reducing sugars.

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Table 2. Means of population and index selected parents.

	n	PMI mg/g	TA %	Sugars %
Population	400	190	2.51	16.2
Selections	60	157	2.88	15.5
Difference	---	-33	0.37	-0.7
% of Pop'n.	---	-17.4	14.7	-4.2

C. Cloning of Nicotiana DNA (with Kathryn Murphy)

One of the major problems in investigating the tobacco mitochondrial genome has been the isolation of clean, restrictable DNA in sufficient quantities for restriction analyses. Many workers use mitochondrial DNA from tissue-cultured cells. We have decided to use plant leaves as our source, however, to avoid problems known to occur in cultured cell lines. By modifications made in existing isolation techniques we are able to obtain, on the average, enough DNA for ten restrictions from one isolation using approximately 200 grams of fresh weight material. This has made investigation of several questions much easier.

The effect of anther culture on the mitochondrial genome has been investigated in two ways. Restriction patterns of twenty-five dihaploids have been compared and found to be identical. Several tobacco DNA clones have been used to probe the above dihaploids to verify that restriction bands in the same location do indeed contain the same sequences. These probes have also been used to examine other dihaplods. The same pattern was always obtained for each dihaploid with a given probe. There is, to date, no indication that anther culture produces large-scale variations in the mitochondrial genome.

In tobacco, cytoplasmic male sterility is obtained by combining the cytoplasm of one species with the nucleus of another. Two such male steriles, Nicotiana tabacum nucleus with a N. suaveolens cytoplasm and N. tabacum nucleus with a N. megalosiphon cytoplasm have been investigated. Earlier work had shown that clones hybridized to restriction digests showed differences in patterns of hybridization between N. megalosiphon and the male steriles identified as having that cytoplasm, although the male steriles showed identical patterns of hybridization. N. suaveolens and the male steriles containing the N. suaveolens cytoplasm showed identical patterns of hybridization. Recently, restriction digests of these different tobaccos have been compared. N. megalosiphon and its putative male steriles were found to differ extensively in their restriction patterns. The differences are so great as to make it highly

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unlikely that the species currently identified as N. megalosiphon was actually the source of the cytoplasm for the male sterile currently bearing that name.

Tobacco mitochondrial RNA has been isolated and used to examine clone banks for potential genes. Several clones containing highly transcribed sequences have been identified in this way. At least two of these sequences have been found to hybridize with corn mitochondrial RNA, as well as tobacco mtRNA. As these clones do not hybridize to any of the known genes currently available as probes in the lab, they have been designated URF's (unassigned reading frames). These and known genes have been hybridized to Northern blots of tobacco and corn mtRNA, and their patterns of transcription noted. One clone, HEIII3A, was found to give only one RNA band in both species, while the other probes (with the exception of the ribosomal genes) gave much more complex patterns, perhaps indicating extensive processing. This clone has been pulled out of corn as well. It is currently being cloned into M13 and will probably be sequenced shortly.

RNA transcription in corn and tobacco has been compared in a superficial way. The large ribosomal RNA is found to be considerably larger in corn than in tobacco, and the 16S RNA is slightly larger in corn. A very bright RNA of approximately 400 bases is found in tobacco, but not in corn. Attempts are underway to find the stretch of DNA which codes for this RNA.

D. Contribution of Somaclonal Variation to Genetic Variability Among Doubled Haploid Lines

Haploid plants were obtained from three highly inbred cultivars and chromosome doubled via in vitro leaf midvein tissue culture to investigate the potential contribution of the chromosome doubling procedure to genetic variability among doubled haploid (DH) lines. Single plants of cultivars 'Coker 139' (C139) and 'NC95' were self pollinated and crossed as the female parent with Nicotiana africana. Haploid plants were identified among the surviving seedlings in these highly lethal interspecific crosses. A single plant of flue-cured cultivar '402' was self pollinated and anthers were cultured to generate anther-derived haploid plants. Segments of leaf midveins from fully-expanded leaves of haploid plants were surface sterilized and cultured on a Murashige-Skoog (MS) regeneration medium supplemented with 2.5 mg/l kinetin and 4.0 mg/l IAA. Regenerated shoots from the cultures were transferred to MS medium without hormones to promote root development and rooted shoots were transplanted to 15 cm clay pots in the greenhouse. DH lines with euploid chromosome numbers (2n=48) were identified among the regenerated plants by first selecting for fertile genotypes, followed by cytological analyses of pollen mother cells. Four doubled-haploid lines arising from cultures of different leaf midvein segments, from each of four haploid plants of each cultivar were identified and self pollinated. DH lines and parental cultivars were evaluated for agronomic and leaf chemical characteristics in two environments. Since comparisons among

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genetic materials from different cultivars were not of great interest, a set structure was employed in the field design, and cultivar effects were confounded with sets. The 16 DH lines and selfed progenies of the original parental plant from which the haploids were derived were grown in three replications in each set. Data were collected on cured leaf yields (kg/ha), days to flower, grade index, number of leaves per plant, plant height (cm) and percentages of reducing sugars and total alkaloids as nicotine in cured leaf. Analyses of variance were conducted for each parameter and variability among treatments within cultivars (sets) was partitioned into an orthogonal set of comparisons of parent versus the DH lines, variation among the four groups of DH lines in each variety and among the DH lines within each DH group within each cultivar.

Mean performance of the DH lines and parental cultivars is shown in Table 3. Anther-derived doubled haploids (ADHs) of 402 were significantly lower yielding and produced lower quality cured leaf than the cultivar. In addition, significant genetic variability was detected among the four 402 ADH groups for all seven parameters measured (Table 4). MDH lines of C139 and NC95 did not differ from the cultivars for any parameter. Variability among the NC95 MDH groups was detected for leaf yields, and percent total alkaloids, however. This was due primarily to the high leaf yields and low alkaloids produced by DH lines in group 2 (Table 4). Significant genetic variability was not found among DH lines within DH groups within cultivars for any character investigated. This suggests that the four doubled haploids arising from different leaf midvein segments from the same haploid plant are not generally different. Hence the contribution (somaclonal variation) to genetic variability among doubled haploid lines from the in vitro chromosome doubling procedure was not important in these materials. Instead, genetic variability among DH lines in the cultivars investigated appears to have arisen from genetic differences among the haploid nuclei which gave rise to the haploid sporophytes.

Table 3. Mean Performance of Parental Cultivars and Doubled Haploid Lines.

Genotype	Yield	Grade Index	Days to Flower	Leaves	Plant Height	Sugar	Total Alkaloids
	kg/ha			no.	cm.	- - - %	- - -
Coker 139	4018	30	67	20.2	140	21.1	1.52
Mean C139 MDHs	3948	33	66	20.0	138	20.7	1.59
NC95	3812	31	66	19.7	140	21.1	2.29
Mean NC95 MDHs	3790	30	65	19.6	140	20.7	2.08
402	3187	33	64	17.7	142	18.3	2.73
Mean 402 ADHs	2823*	24**	62	16.9	142	16.7	2.52

*, ** Significantly different from parental cultivars at 0.05 and 0.01 probability, respectively.

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Table 4. Mean Performance of Parental Selfs and Four Doubled Haploid Groups of Three Cultivars

	Yield	Grade Index	Days to Flower	Leaves	Plant Height	Sugar	Total Alkaloids
	kg/ha			no.	cm.	- - - %	- - -
Coker 139 Family							
C139 139 (Parent)	4018	30	67	20.2	140	21.1	1.52
C139 MDH Group 5#	3893	32	66	20.1	142	20.8	1.58
C139 MDH Group 11	3915	34	65	19.5	134	21.1	1.66
C139 MDH Group 14	3997	32	66	19.9	138	20.5	1.61
C139 MDH Group 18	3988	34	66	20.2	139	20.3	1.53
L.S.D. 0.05	ns	ns	ns	ns	ns	ns	ns
NC95 Family							
NC95 (Parent)	3812	31	66	19.7	140	21.1	2.29
NC95 MDH Group 2#	4177	28	66	20.0	141	20.8	1.50
MC95 MDH Group 4	3608	31	66	19.9	141	21.1	2.21
NC95 MDH Group 5	3586	31	65	18.9	137	20.5	2.21
NC95 MDH Group 6	3787	30	64	19.5	140	20.3	2.40
L.S.D. 0.05	201	ns	ns	ns	ns	ns	ns
402 Family							
402 (Parent)	3187	33	64	17.7	142	18.3	2.73
402 ADH Group 1#	3010	24	65	17.2	150	16.4	1.94
402 ADH Group 2	2377	21	60	15.4	129	14.7	2.42
402 ADH Group 3	2981	28	63	17.5	145	18.1	2.81
402 ADH Group 4	2924	25	63	17.5	147	17.8	2.91
L.S.D. 0.05	2.01	3.2	1.9	0.9	5.5	1.6	.28

Each DH group is the mean of four DH lines.

E. Development of flue-cured and burley tobacco germplasm with tolerance to blue mold. Dr. Rebecca C. Rufty

The objectives of our program are to characterize genetic mechanism(s) for blue mold resistance in tobacco cultivars of world-wide origin; to determine if the chromosomal sites of interspecific gene-incorporation for blue mold resistance are similar in cultivars emanating from different interspecific crosses, and to develop burley and flue-cured tobacco breeding lines which might combine multiple genes for disease resistance in F_1 hybrid form. Such F_1 hybrids might possess adequate levels of protection from the disease without serious yield and quality reductions generally associated with interspecific transfers in homozygous form.

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Blue mold resistant cultivars Z-3 and Ovens 62 were initially hybridized with susceptible flue-cured and burley cultivars McNair 944, NC 95, NC 13, Ky 15 and Ky 17. Genetic materials were generated from F_1 hybrids derived from the above crosses using the anther culture procedure. Two approaches have been utilized to reach the objectives of this program: inoculations at both the haploid and the dihaploid level.

In the first approach, haploid plants arising from F_1 hybrids of resistant x susceptible crosses were inoculated with Peronospora tabacina under greenhouse conditions. In this procedure, segregation is among individuals and since every genotype is unique, no replication is possible. Nevertheless, chromosome doubling by leaf midvein tissue culture is performed only on selected individuals and this minimizes the labor-intensive procedures commonly used to restore fertility. Results from these experiments are shown in Table 5.

Table 5. Selection of anther-derived haploid plants resistant in Peronospora tabacina from crosses of resistant x susceptible cultivars.

Cross	Haploids Tested	Resistant Haploids Selected	Selected
	- - - - - - no. - - - - - -		%
Z-3 x McNair 944	152	21	14
Z-3 x NC 95	147	8	5
Ovens 62 x Ky 15	268	12	4
Ovens 62 x Ky 17	161	23	14
Ovens 62 x McNair 944	130	27	21
Ovens 62 x NC 13	99	1	1
TOTAL	957	92	10

Considerable difficulty was encountered in obtaining moderate, uniform levels of disease development in greenhouse experiments. Environmental and error variability associated with greenhouse inoculation, and disease development on individual plants, appear to be sufficiently great that the genotype of an individual plant may not be accurately determined. Hence, determination of the underlying genetic basis for disease resistance in F_1 hybrids of resistant x susceptible cultivars by studies of segregating ratios on individual haploid plants

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may not be feasible. Therefore, a second approach was used to assess resistance reactions. In this procedure, approximately 4000 haploid plants were first treated with colchicine to double their chromosome number generating almost 600 dihaploid lines. Multiple plants from any doubled haploid can be inoculated to discern the disease reaction of a family and field tests can be carried out under multiple environments to improve genetic precision.

From the germplasm pool generated by the two procedures, fifty dihaploid lines selected for blue mold resistance under greenhouse conditions and 350 unselected dihaploid lines were tested at the Estacion Experiment de Gurabo, Gurabo, Puerto Rico during the 1983-84 winter season. Check cultivars and selected and unselected dihaploids were assigned to sets and planted in two replications of five plant plots. Fertile and cytoplasmic male-sterile commercial cultivars were planted every third row to serve as disease spreaders and to obtain information on their disease reaction.

Results from this experiment have not been statistically analyzed and evaluations may be inconclusive due to poor blue mold development. Nevertheless, the following observations have been made:

1. There appear to be genetic differences for blue mold resistance among dihaploid lines.

2. Certain entries appeared to be as resistant to Peronospora as the resistant parents and yet possessed superior agronomic characteristics.

3. Genotypes resistant to Potato Virus Y (PVY) derived fron Virgin A Mutant (VAM) were much more susceptible to Blue Mold than PVY-susceptible lines. Similar results were found in 1982-83. Care must be taken in releasing materials with the VAM-type resistance due to their increased vulnerability to Peronospora.

4. The blue mold reaction of male-sterile cultivars was very similar to that of their fertile counterparts indicating that the alien cytoplasm conditioning male sterility had no effect on disease development. Thus, cytoplasmic male-sterile females can be used in developing blue mold resistant F_1 hybrids.

Selected materials need to be retested under North Carolina conditions and preferably under higher disease pressure to increase the level of confidence placed on genotypic responses to blue mold.

In 1983, the following crosses were made among the various sources of resistance to blue mold: Z-3 x Ovens 62, Ovens 62 x Sirone, Ovens 62 x Sirogo, Ovens 62 x Florida 513, and Sirone x Sirogo. F_1 hybrids of resistant x resistant cultivars were anther-cultured and 150-200 haploid plants were produced from each. Haploids were treated with colchicine to double their chromosome numbers and obtain dihaploid lines.

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These fertile lines will be inoculated with Peronospora in 1984 to determine if segregation for disease resistance will occur. Such experiments will establish whether blue mold resistant genes are similar in cultivars emanating from different interspecific crosses. Should the genes for resistance be different, lines with combined resistance from the different sources can be developed. If the genetic systems conditioning resistance are the same, it is possible for these genes to be incorporated into different chromosomal sites. Resistance genes in homozygous condition are associated with poor yield and agronomic quality. A high level of resistance may be obtained by having multiple doses of the same gene(s) in heterozygous condition. Therefore, hybrid lines with duplicate doses of the same resistance factor(s) would be developed.

II. Graduate Students:

Bland, Molly M., M.S. Candidate, Genetics. Molecular genetics of tobacco.

Corbin, T.C., Ph.D. Candidate, Crop Science. A comprehensive tobacco breeding program utilizing gynogenic haploids.

Schnell, R.J.II, Ph.D. Candidate, Crop Science. Anther culture induced genetic changes as a source of variability for tobacco improvement.

Witherspoon, William D. Jr., Ph.D. Candidate, Crop Science. Evaluation of two forms of recurrent selection in the separate genomes of amphidiploid Nicotiana tabacum L.

III. Postdoctoral Fellows:

Murphy, S. Kathryn. Cloning of Nicotiana DNA.

Rufty, Rebeca C. Development of flue-cured and burley tobacco germplasm with tolerance to blue mold.

IV. Publications:

Brown, J.S., E.A. Wernsman and R.J. Schnell, II. 1983. Effect of a second cycle of anther culture on flue-cured lines of tobacco. Crop Sci. 23:729-733.

Dhillon, S.S., E.A. Wernsman and J.P. Miksche. 1983. Evaluation of nuclear DNA content and heterochromatin changes in anther-derived dihaploids of tobacco (Nicotiana tabacum) cv. Coker 139. Can. J. Genet. Cytol. 25:169-173.

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Rufty, R.C., E.A. Wernsman and N.T. Powell. 1983. A genetic analysis of the association between resistance to Meloidogyne incognita and a necrotic response to infection by a strain of potato virus Y in tobacco. Phytopathology 73:1413-1418.

Van Sanford, D.A. and D.F. Matzinger. 1983. Ten cycles of mass selection for tobacco seedling weight under two nutrient regimes. Crop Sci. 23:1163-1167.

V. Manuscripts Accepted for Publication:

Beatson, R.A., E.A. Wernsman and R.C. Long. Divergent mass selection for carotenoids in a flue-cured tobacco population. (Accepted by Crop Science).

Bowman, D. T., E. A. Wernsman, T. C. Corbin and A. G. Tart. Contribution of genetics and production technology to long term yield and quality gains in flue-cured tobacco. (Accepted by Tobacco Science).

Matzinger, D.F. and L.G. Burk. Cytoplasmic modification by anther culture in Nicotiana tabacum L. (Accepted by Journal of Heredity).

VI. Papers Presented at Professional Meetings:

Reed, S.M. and E.A. Wernsman. The doubled haploid experience in Nicotiana. Annual Meeting of the Crop Science Society of America. Washington, D.C. August, 1983.

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PLANT PATHOLOGY

STATE 3572: EPIDEMIOLOGY AND CONTROL OF VIRUS DISEASES OF SOLANACEOUS CROPS

G. V. Gooding, Jr., Project Leader

I. SUMMARY OF RESEARCH

An investigation of the strains of potato virus Y that occur on tobacco in various countries was completed. Five distinct strains are now known to occur in the United States. Two cause mild and three severe (necrotic) symptoms. In addition, three naturally occurring isolates from the U.S. were identified that cause necrosis on NC 744, a breeding line that originated from Virgin Mutant A. Necrotizing strains were also identified from Argentina, Chile, Europe, South Africa and South Korea. All strains are serologically identical except the Argentine strain. The Argentine strain is serologically related to, but antigenically distinct from the rest of the group. Strains were differentiated using different sources of tobacco germplasm. The information gained in this study will be used in selecting germplasm for the development of resistance and the deployment of resistant cultivars. For example, Havana 307 was found resistant to most of the necrotizing strains, all of those from the U.S., and is being used by Drs. Wernsman, Chaplin, and Sisson to develop a PVY resistant flue-cured breeding line or cultivar.

Knowledge of the identity, distribution, and incidence of viruses is fundamental to the development of disease control strategies. Data on virus distribution and incidence must be based on a statistically sound sampling procedure if confidence levels are to be assigned, however. A three-year study was completed in 1983 to determine virus distribution and incidence in North Carolina using randomly selected farms in each of nine countries which serve as epicenters for the surrounding tobacco production area. The data from this study are summarized in Table 1.

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Table 1. Occurrence of tobacco viruses in North Carolina in 1981, 1981 and 1983.[a]

Type	Year	Virus incidence (%)			
		TMV[d]	PVY[d]	TEV[d]	TVMV[d]
Burley[b]	1981	0	0	0	33.8
	1982	0	0	0	4.3
	1983	0	0	0	7.8
Flue-cured[c]	1981	7.3	0	0.8	0
	1982	6.0	0.2	0.1	0
	1983	4.6	0.2	0	0

[a]Data from 4 randomly selected farms in each county. Data was obtained from the same farms and counties each year.

[b]Haywood and Watauga counties.

[c]Stokes, Granville, Bertie, Wayne, Pamlico, Columbus, and Montgomery counties.

[d]TMV=tobacco mosaic virus; TEV=tobacco etch virus; TVMV=tobacco vein mottling virus; PVY=potato virus Y; TRSV=tobacco ringspot virus.

No tobacco etch virus (TEV) was detected on burley tobacco in 1981, 1982 or 1983 based on the data in Table 1. We know based on other work there was some TEV, as well as other viruses, on the burley crop. These data, of course, simply mean that viruses other than TVMV were of too low incidence to be detected using this sampling procedure. When these data are analyzed by Drs. L. A. Nelson and C. H. Proctor, with whom I have been cooperating on this work, detection levels with confidence limits will be ascribed.

Cross protection studies were continued using the mild isolate of potato virus Y from U.S. potatoes and a necrotizing isolate from Chile. The cross protecting ability of the mild isolate was found temperature and cultivar independent but necrotizing strain concentration dependent. Nicotiana longiflora was identified as a local-lesion host of the Chile strain but not the potato strain. I now have the system to determine if cross-protection with these strains is based on prevention of

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infection by the Chile strain, limiting replication or movement of the Chile strain and other basic studies. Trials are underway in Chile to determine if cross-protection is of practical value for control.

Unless there is some factor of which I am unaware, the primary source of tobacco mosaic virus for tobacco is infested debris from the previous year's crop. Several chemicals that have the potential for preventing infection at transplanting have been identified and will be tested for this purpose in field trials in 1984.

II. PUBLICATIONS

Johnson, C. S., Main, C. E., and Gooding, G. V. , Jr. 1983. Crop loss assessment for flue-cured tobacco infected with tobacco mosaic virus. Plant Dis. 67:881-885.

Mercer, D. W., Gooding, G. V., Jr., and Kennedy, G. C. 1982. Transmission of tobacco mosaic virus by Epitrix hirtipennis. Tobacco Sci. 26:129-132.

Powell, N. T., and Gooding, G. V., Jr. 1983. Control of tobacco mosaic virus on flue-cured tobacco. Plant Path. Inf. Note 203.

Rufty, R. C., Powell, N. T., and Gooding, G. V., Jr. 1983. Effect of aerial and soil temperature on disease severity in tobacco inoculated with potato virus Y and Meloidogyne incognita. Phytopathology 73:1418-1423.

III. MANUSCRIPTS ACCEPTED FOR PUBLICATION

Spurr, H. W., Jr., Gooding, G. V., Jr., and Todd, F. A. 1984. An investigation of metalaxyl soil treatment for tobacco blue mold control on the incidence and development of tobacco mosaic virus and Granville wilt. Tobacco Sci. (In press)

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Source: https://www.industrydocuments.ucsf.edu/docs/xtyk0000

Title: Soilborne Diseases of Tobacco

Project Leader: H. D. Shew

I. Summary of Research:

Black Shank.- The influence of soil water matric potential on production of sporangia of Phytophthora parasitica var. nicotianae was determined. The optimum matric potentials for sporangial production differed with soil type, but generally were between -40 and -200 mb (-0.04 to -0.2 bar). Sporangial production was more abundant and formed over a wider range of matric potentials in soil conducive to development of black shank than in a soil suppressive to black shank.

Soil material representing 12 soil types was collected and screened in the greenhouse for black shank development. Soil material ranged from very conducive to suppressive to black shank. From this study, two suppressive and two conducive soil materials were chosen for study under controlled environmental conditions in the phytotron. Soil matric potentials of -10, -20, -40 and -40 plus a 24 hr saturation period were imposed on each soil. Disease was most severe in the conducive soils. Possible explanations for the suppressive nature of some soils include antagonistic microflora and the chemical status of the soils. The relationship between soil flooding, host resistance and the number of pathogen zoospores required to kill the seedlings was also determined.

Isolates of P. parasitica var. nicotianae were collected after 2 or 3 years of consecutive use of the fungicide metalaxyl. In several fields there was a gradual shift in sensitivity to metalaxyl in the pathogen population. Almost 900 isolates have been screened in vitro to determine range in sensitivity to metalxyl in P. parasitica var. nicotianae (Table 1) and to observe long term effects of metalaxyl on the pathogen (Table 2). An in vivo screening procedure was also developed.

An experiment was conducted for the second year in microplots at the Upper Coastal Plain Research Station on the influence of root knot nematodes and host resistance on control of black shank by metalaxyl. The use of metalaxyl increased nematode populations for the second year (Table 3). Metalaxyl effectively controlled black shank in the absence of root knot nematodes on the nematode susceptible cultivar 'coker 48' and in the presence of root knot nematodes on the nematode resistant cultivar 'Speight G-28'. Host resistance alone did not control black shank and allowed high populations of the fungus pathogen buildup in the soil.

Small plot tests were also repeated for the second year to determine if P. parasitica var. nicotianae spreads from a point source of inoculum under field conditions in the absence of

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cultivation. The summer of 1983 was very dry and at no time during the season was standing water present in the fields. Only on the highly susceptible 'Hicks' was spread detected. However, soil assays taken at predetermined distances from the point source of inoculum indicated that the inoculum had moved in all rows assayed.

Six different solanaceous crops were inoculated with zoospores of P. parasitica var. nicotianae to determine if the black shank fungus will infect and survive in plants other than tobacco. All six species were infected under greenhouse conditions. The significance of these infections in survival of the pathogen is being determined.

Granville Wilt.- Studies were continued on the infleunce of soil temperature, water stress, and host resistance on development of wilt. Drought stress delayed onset of sysmptoms whereas high temperatures and high soil moisture encouraged disease development.

II. Graduate Students

Jill R. Sidebottom, M. S., Plant Pathology. "Influence of Soil Matric Potential and Soil Type on Development of Tobacco Black Shank."

IV. Publications:

Shew, H. D. 1983. Effects of soil matric potential on infection of tobacco by Phytophthora parasitica var. nicotianae. Phytopathology 73:1160-1163.

VII. Papers Presented:

Shew, H.D. "Effect of Fungicides in Phytophthora Selective Media on Germination and Colony Formation by Zoospores of Phytophthora parasitica var. nicotianae." Annual meeting of The American Phytopathological Society, Ames, IA. June 26-30, 1983.

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Table 1. Mean growth and percent inhibition of *Phytophthora parasitica* var. *nicotianae* in metalaxyl incorporated agar.

Metalaxyl conc. (ppm)	Growth[x,y] (mm)	% Inhibition
0	18.6	-
0.1	13.2	29.0
1.0	7.3	60.8
10.0	1.8	90.3
100.0	1.6	91.2

[x]Mean of 857 isolates with three replications per isolate.

[y]Mean growth on 5% V-8 juice agar after 7 days in the dark at 23-25C.

Table 2. Percent inhibition of growth of *P. parasitica* var. *nicotianae* in metalaxyl incorporated agar[x].

Conc (ppm)	Years of Exposures			
	0	1	2	3
0.1	31	34	20	22
1.0	63	71	60	42
10.0	91	90	90	88
100.0	91	90	93	92

[x]Mean inhibition of all isolates collected. Total number of isolates: Year 0 =317; Year 1 = 271; Year 2 = 124; and Year 3 = 145.

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Table 3. Influence of metalaxyl on final populations of root knot larvae per pint of soil.

Treatment	Coker 48		Speight G-28	
	1982	1983	1982	1983
N[x]	4410	1448	1336	2030
N1	4509	3858	1360	3130
N2	6292	3994	1635	528
NF	4127	1616	651	814
NF1	5524	434	980	1636
NF2	4824	550	1218	1705

[x]N = Nematode alone; N1 = nematodes plus 1 lb metalaxyl; N2 = nematode plus 2 lb metalaxyl; NF, NF1, and NF2 also have the fungus (F) present in the soil

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Title: NC 03429 Breeding for Disease Resistance in Tobacco

Project Leaders: N. T. Powell and Margaret E. Daub

I. Summary of Research:

This project has the following objectives:

A. To develop superior disease resistant tobacco cultivars.

B. To develop combinations of genetic materials which can be used in both public and commercial breeding programs designed to produce tobacco cultivars.

C. To seek and develop additional sources of genetic resistance to tobacco diseases.

Over 500 breeding lines were evaluated during 1983 for yield, quality, other agronomic features, and disease resistance. These investigations, conducted on 6 research stations, included lines in various stages of development and genetic stability, ranging from the F_2 to the F_{10} generations. Disease resistances emphasized were black shank, root knot, Granville wilt, mosaic, brown spot, Fusarium wilt and the potato virus Y. A new multiple resistant cultivar, NC 567, will be available to farmers from this program in 1984. NC 567 has resistance to black shank, Granville wilt, root knot, mosaic and the tobacco cyst nematode. Of several lines tested in 1983 Official Variety Tests, one (NC 2513) has been advanced to regional testing in 1984. In addition, 2 advanced lines were chosen for entry into 1984 Official Variety Tests. All of these advanced lines carry multiple disease resistance. Several hundred individual plants were selected in 1983 for further evaluation. Selections were based on plant type, yield, leaf quality characteristics, and resistance to multiple diseases. The best of these selections will be tested in 1984 in breeding plots on five research stations for yield, quality characteristics, and resistance to black shank and Granville wilt. In addition, they will be screened in the greenhouse for root knot nematode and mosaic resistance. The program to develop superior burley breeding lines with disease resistance was continued. Several advanced lines will be tested in 1984 Official Variety Tests. New hybrids were made in 1983 and will be evaluated during the 1984 season.

IV. Publications:

Campbell, C. L., W. R. Jacobi, N. T. Powell, and C. E. Main. 1984. Analysis of disease progression and plants during tobacco black shank epidemic. Phytopathology 74:230-235.

IX. Acknowledgements:

We wish to thank Mr. D. Wayne Pollard for excellent technical assistance.

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Title: Transfer of Resistance Genes from Nicotiana repanda to Nicotiana tabacum by protoplast fusion

Project Leader: Margaret E. Daub

I. Summary of Research:

A. Objectives

1) To develop a protoplast fusion technique for generating hybrids between Nicotiana repanda and commercial cultivars of flue-cured and burley tobacco.

2) To use tissue culture techniques to attempt to stabilize the hybrids.

3) To initiate a backcross program in order to generate disease-resistant germplasm for use in a breeding program.

B. Progress Report

A project has been initiated to use protoplast fusion techniques to transfer disease-resistance genes from a wild Nicotiana species, N. repanda, to high quality cultivars of flue-cured and burley tobacco. N. repanda is of particular interest for two reasons. First of all, it carries resistance to more tobacco diseases than any other Nicotiana species. These include anthracnose, black root rot, black shank, brown spot, frogeye leaf spot, powdery mildew, tobacco rattle virus, mosaic, wildfire, and root knot and cyst nematodes. Of critical importance here is the root knot nematode resistance, for N. repanda is resistant to Meloidogyne javanica and M. arenaria as well as the common species, M. incognita. These two nematode species have become an increasingly serious problem in North Carolina, and current sources of root-knot nematode resistance are susceptible to them. Secondly, genes from N. repanda have not yet been incorporated into tobacco cultivars due to the difficulty of crossing N. repanda and N. tabacum by conventional methods.

Progress is being made in the development of a protoplast fusion system. A semi-dominant albino marker gene (Su), presently found in a broad-leaf tobacco cultivar, is being crossed into eight high-quality flue-cured and burley cultivars (NC2326, NC82, Coker 319, McNair 944, K-399, Speight G-28, Speight G-70, and Burley 21). This marker gene will be used to select the hybrid fusion products from unfused protoplasts. Hybrid lines have been back-crossed twice to the flue-cured and burley cultivars; six back-cross steps are required in order to incorporate approximately 99% of the back-cross parental genes. Conditions have also been established for obtaining rapidly-growing, friable callus and suspension cultures of the eight

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cultivars; these cultures are required in order to maintain albino tissues of the cultivars. Cultures of all eight tobacco cultivars grow well on a basic Murashige and Skoog medium with 3 mg/l Indoleacetic acid, 0.3 mg/l Kinetin and 50 μM IAA-L-alanine. Current efforts are directed toward developing techniques for the isolation of protoplasts from N. repanda and from hybrids of N. repanda and N. sylvestris. Standard techniques developed for protoplast isolation from N. tabacum proved to be inadequate for N. repanda and the N. repanda - N. sylvestris hybrid. The use of a more active macerase and stronger sterilizing procedures have resulted in satisfactory isolation of sterile, healthy protoplasts. Current efforts are directed toward the development of improved nutrient media in order to regenerate plants from these protoplasts.

IV. Acknowledgements:

This work was supported in part by a grant from the North Carolina Tobacco Foundation.

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Title: The Role of Photosensitizing Fungal Toxins in Plant Disease

Project Leader: Margaret E. Daub

I. Summary of Research

A. Objectives

1) To determine the mode of action of the photosensitizing phytotoxin cercosporin on tobacco cells.

2) To identify resistance mechanisms to cercosporin.

B. Progress Report

Cercosporin is a phytotoxin produced by members of the genus Cercospora, a large group of fungal plant pathogens which cause damaging diseases on a large number of host species worldwide. Cercosporin is unique among the plant pathogen toxins because it is a photosensitizing compound; in the presence of light, the cercosporin molecule is converted to an electronically excited state which then reacts with oxygen to produce activated oxygen species which are toxic to living cells. We have recently demonstrated that cercosporin, when illuminated by light, generates both singlet oxygen and superoxide in vitro. However, singlet oxygen appears to be the primary mechanism of cercosporin activity in vivo. First of all, quenchers of singlet oxygen prevent the killing of tobacco cells by cercosporin. Secondly, tobacco cells which are resistant to the superoxide-generating herbicide paraquat and which have elevated levels of oxidative enzymes are not resistant to cercosporin, suggesting that superoxide is not important in in vivo toxicity.

Tobacco cell death appears to be due to a cercosporin-mediated peroxidation of the membrane lipids. We have shown that cercosporin-treatment of cells results in a marked increase in the percent of saturated to unsaturated fatty acids, and both a decrease in cell membrane microviscosity and an increase in the membrane phase transition temperature. These changes make the tobacco cell membranes very leaky, and it is hypothesized that this provides the pathogen (which is limited to growth within the intercellular spaces of the leaf) with the nutrients required for growth and sporulation in the host. These changes are also consistent with ultrastructural observations made on Cercospora-infected plants.

Repeated attempts to select plant cell and protoplast cultures for resistance to cercosporin have failed. This result is not surprising, for singlet oxygen is an extremely toxic compound to which few organisms are resistant. The fungus, however, produces high concentrations of cercosporin in the light and is apparently unaffected by it. This resistance appears to

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be a fairly general phenomenon in fungi, for a number of other fungi have also been found to be resistant to cercosporin. Current efforts are being directed toward elucidating the mechanisms of resistance of fungi to cercosporin. The goal of this project is to ultimately identify and characterize genes for resistance to cercosporin, and to other singlet oxygen-and free radical-generating agents in general. This work will serve as a basis for future research on the isolation and cloning of cercosporin-resistance genes.

II. Graduate Students:

Kimberly D. Gwinn

IV. Publications:

Daub, M. E., and Briggs, S. P. 1983. Changes in tobacco cell membrane composition and structure caused by cercosporin. Plant Physiol. 71:763-766.

Daub, M. E., and Hangarter, R. P. 1983. Light-induced production of singlet oxygen and superoxide by the fungal toxin, cercosporin. Plant Physiol. 73:855-857.

V. Manuscripts Accepted for Publication:

Daub, M. E. 1984. A cell culture approach for the development of disease resistance: studies on the phytotoxin cercosporin. Hort. Sci. 19:(In press).

IX. Acknowledgements:

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